☐ Please charge my Deposit Account No. 08-0380 in the amount of $_____.

☐ A check in the amount of $_____ is attached.

☒ A separate Petition for Extension of Time is being filed concurrently herewith.

☒ Payment for the extension fee is included with the petition.

☐ Deposit Account No. 08-0380 is being charged for the extension fee.

☒ The Commissioner is hereby authorized to charge payment of the following fees associated with this communication or credit any overpayment to Deposit Account No. 08-0380.

☒ Any filing fees under 37 C.F.R. 1.16 for the presentation of extra claims.

☒ Any patent application processing fees under 37 C.F.R. 1.17.

Any extensions of time that are required to maintain this application in a pending status, if not included herewith, are hereby requested. The Commissioner is hereby authorized to charge such extension fees to Deposit Account No. 08-0380. Two copies of this transmittal letter are enclosed for accounting purposes.

Respectfully submitted,

HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

Richard W. Wagner

By Richard W. Wagner
Registration No. 34,480
Agent for Applicant(s)
(617) 861-6240

Dated: September 23, 1991

SPC89-05 Amend B
RWW:s
9/23/91
RWW/pah

PATENT APPLICATION
Docket: SPC89-05

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Timothy J. Barberich and James W. Young

Serial No: 07/461,262 Group Art Unit: 125

Filed: January 5, 1990 Examiner: L. Schenkman

Title: METHOD FOR TREATING ASTHMA USING OPTICALLY PURE R(-) ALBUTEROL

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to Honorable Commissioner of Patents and Trademarks, Washington, D.C. 20231, on 9/23/91.

Hamilton, Brook, Smith & Reynolds

B. J. Hamilton
Signature

9/23/91
Date

AMENDMENT B

The Honorable Commissioner
of Patents and Trademarks
Washington, D.C. 20231

Sir:

Applicants' Agent respectfully requests a three-month extension of time to reply to the Office Action mailed from the Patent Office on March 22,

1991. A separate Petition for Extension of Time and the appropriate fee are being filed concurrently.

Responsive to the Office Action mailed from the Patent Office on March 22, 1991, please amend the above-identified application as follows:

In the Claims

In Claim 2, line 3, after "weight" insert ---of total albuterol---.

In Claim 3, line 3, after "weight" insert ---of total albuterol---.

6. (Amended) A method of treating asthma in an individual with albuterol, while reducing side effects associated with albuterol, comprising administering to the individual a quantity of an optically pure R(-) isomer of albuterol sufficient to result in bronchodilation and at least one additional drug selected from the group consisting of bronchodilators, antihistamines and analgesics.

Cancel Claim 7.

In Claim 8, line 1, change "Claim 7" to ---Claim 6---.

9. (Twice Amended) A composition comprising a mixture of ~~an optically pure~~ R(-) isomer of albuterol and at least one other drug selected from the group consisting of bronchodilators, antihistamines and analgesics.

Cancel Claims 10, 11 and 12.

Add the following new Claims:

13. A composition of Claim 9 wherein the amount of the R(-) isomer of albuterol is greater than approximately 90% by weight of total albuterol.

B3

14. A composition of Claim 13 wherein the amount of the R(-) isomer of albuterol is greater than approximately 99% by weight of total albuterol.

REMARKS

Rejection of Claims 1-12 under 35 U.S.C. §103

Claims 1-12 have been rejected under 35 U.S.C. §103 over Chemical Abstracts which, it was stated, teaches salbutamol (albuterol) used to treat asthma and compositions containing albuterol. It was further stated that the determination of a particular isomer would be a matter of obvious alternatives. Finally, it was stated that difference in activity between isomers is not unexpected (In re Adamson et al.).

Applicants respectfully traverse this rejection. The Chemical Abstracts reference shows the bronchodilator effects of salbutamol and drug combinations incorporating salbutamol. This reference does not teach nor suggest the use of an optically pure isomer of salbutamol, either alone or in combination with other drugs. From this reference, a person of ordinary skill in the art would not be

motivated to use an optically pure isomer of salbutamol to result in bronchodilation because there is no suggestion of the efficacy of such an isomer in the reference.

In re Adamson et al. does not cure this defect in the Chemical Abstracts reference. In re Adamson et al. teaches that optical isomers, or methods of separating such isomers, of compounds that are art recognized as having optical isomers are unpatentable. In re Adamson et al. indicates that physiological behavior of the stereo-isomers can differ considerably. However, In re Adamson et al. is not directed to the patentability of methods of eliciting physiological responses, such as treating asthma, of optically pure isomers. More importantly, neither the Chemical Abstracts reference nor In re Adamson et al. teaches or suggests the use of R(-) albuterol to result in bronchodilation. Applicants' disclosure teaches this method. The Chemical Abstracts reference does not indicate or suggest the use of the R(-) isomer of salbutamol and In re Adamson et al. does not teach the efficacy of this isomer but, rather, at best, that this isomer will have a different physiological behavior than the S(+) isomer. For example, from In re Adamson et al., it could be inferred that the physiological property of the R(-) isomer is a toxic effect. Applicants' disclosure, instead, teaches the bronchodilation efficacy of this isomer.

Rejection of Claims 1-5 under 35 U.S.C. §103

Claims 1-5 have been rejected under 35 U.S.C. §103 as being unpatentable over Brittain et al., Hartley et al., Hawkins et al. and Buckner et al, who, it was stated, teach compositions containing the claimed compounds and its isomers used as a bronchodilator in the treatment of asthma. It was further stated that the references teach greater bronchodilation activity of the R(-) isomer over the S(+) isomer, so compositions containing namely the R(-) isomer in the treatment of asthma is clearly rendered obvious by the prior art.

Applicants respectfully traverse this rejection. Brittain et al. show that both isomers and the racemic mixture of salbutamol act on beta$_2$ receptors rather than the beta$_1$ receptors. The (-) isomer and the racemic mixture are roughly equipotent against bronchospasm in guinea pigs (see page 145, last full paragraph) and isolated guinea pig trachea (see page 146, Table 1 and last full paragraph). That is, Brittain et al. show that there is no significant difference in bronchoactivity between (-) salbutamol and the racemic mixture of salbutamol.

Similarly, Hartley et al. show that both isomers and the racemic mixture of salbutamol act on the beta$_2$ receptors rather than the beta$_1$ receptors. The effects of the (-) isomer and the racemic mixture are equiactive on the beta$_2$ receptors of the intact trachea of the guinea pig (see the next to last paragraph of the second column on page 895). Indeed, Table I indicates the racemate of salbutamol is

somewhat more active than the (-) isomer; the mean equipotent doses of racemic and (-) albuterol were reported in Table I to be 4.3 and 6.6, respectively..

Hawkins et al. characterize this study of Hartley et al. by stating that Hartley et al. "reported that racemic salbutamol was 1.5 times as active as the more active (levo) of the two enantiomers." Hawkins et al., in their study, show that the (-) isomer of salbutamol is more active than the racemic mixture when applied against guinea pig tracheal chains (see page 857, top of left column) -- a result that is clearly at odds with the findings of Hartley et al.

Buchner et al. show that the (-) and (+) isomers of salbutamol are more active on guinea pig tracheal strips than on guinea pig atria. There may be more potency for the (-) isomer than the (+) isomer on the tracheal strips, but there is no indication of the potency of the (-) isomer of salbutamol compared with the racemic mixture. This reference is silent concerning the relative efficacy of the (-) isomer and the racemic mixture.

The study by Buckner et al. attempts to ascertain whether the ratio of activity of albuterol (salbutamol) and other beta-agonists towards isolated trachial strips and isolated right atria (in both cases from guinea pig) is dependent on the stereochemistry of the tested drug. These investigators summarize their results as follows (see the right-hand column on page 619): "Even though the potencies of single isomers may differ as much as 24-fold (for salbutamol) between atria and trachea, the stereoselectivity for production of activity is

the same." That is, the ratio of tracheal-to-atrial activities found by Buckner and Abel for the (-) and (+) isomers of albuterol were identical.

These references can be interpreted as indicating that the (-) isomer of salbutamol may not be as effective as the racemate. Since there is lack of agreement between these references concerning the relative efficacy of the (-) isomer and the racemate, a person of ordinary skill in the art would be, at least, confused by these references. For example, if Brittain et al. or Hartley et al were considered, there would be no apparent difference in efficacy between the (-) isomer and the racemate.

In contrast, Applicants' invention teaches the use of R(-) albuterol rather than the racemate to result in bronchodilation. By such administration of the R(-) isomer, the undesirable side effects associated with the racemate are also reduced. Applicants' invention clearly distinguishes over the prior art by specifying the R(-) isomer, rather than the racemate or the S(+) isomer, to result in bronchodilation and to reduce undesirable side effects associated with beta-adrenergic drugs.

Rejection of Claims 6-12 under 35 U.S.C. §103.

Claims 6-12 have been rejected under 35 U.S.C. §103 as being unpatentable over the references cited in the rejection of Claims 1-5 in further view of the Chemical Abstracts reference which shows combinations of drugs, including salbumatol, used in the treatment of asthma.

Applicants respectfully traverse this rejection. The Chemical Abstracts reference does not cure the above discussed shortcomings of the prior art in indicating the

use of R(-) albuterol to result in bronchodilation. The Chemical Abstracts reference does not show the existence or indicate a use for R(-) albuterol. There is no teaching or suggestion in the Chemical Abstracts reference that would motivate a person of ordinary skill in the art to use R(-) albuterol alone or in combination with other drugs in the treatment of asthma. The cited references do not show nor suggest a combination of drugs that include R(-) albuterol to result in bronchodilation.

Rejection of Claims 6 and 9-11 under 35 U.S.C. §112, second paragraph.

Claims 6 and 9-11 have been rejected under 35 U.S.C. §112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter of the invention. It was stated that there is no basis in Claim 9 for the mixture of isomers set forth in Claims 10 and 11. That is, Claim 9 is incorrect in not including the R(-) isomer. It was also stated that Claims 9-12 are too broad absent proportions of ingredients. It was stated that the term "additional drug" in Claims 6 and 9-11 is too broad.

In response to this rejection, Claims 2, 3, 6, 8 and 9 have been amended, Claims 7 and 10-12 have been cancelled and Claims 13 and 14 have been newly added. The amendments to Claims 6 and 9 state the constituency of the "additional drug". Since this constituency had been stated in Claims 7 and 12, these latter claims have been cancelled. Claim 8 was amended to state the proper dependency. Claim 9 was also amended to state the R(-) isomer of albuterol so that Claims 10 and 11 (now Claims

13 and 14) now have proper basis. Claims 2 and 3 were amended by adding the phrase "of total albuterol" to more distinctly claim the subject matter of the invention. Support for this amendment can be found on page 3, lines 25-30 of the specification. Finally, Claims 10 and 11 have been cancelled and replaced by Claims 13 and 14, respectively. These newly added claims are restatements of cancelled Claims 10 and 11 in better grammatical format and are in accordance with Claims 2 and 3.

Applicants respectfully traverse the rejection of Claims 9-12 (now Claims 9, and 13-14) as too broad absent proportions of ingredients. Such proportions are dependent on a number of factors known to a person of skill in the art. These factors include, for example, the individual's age, body proportions, type of disease, severity of symptoms and mode of administration (see the present specification page 4, line 22-page 5, line 2). For this reason, the proportions of ingredients cannot be stated with certitude for all individuals but, rather, must be determined on an individual basis. Skilled artisans determine the ingredient proportions based, at least in part, on the above-listed factors.

CONCLUSIONS

With the above amendments and for the above stated reasons, Applicants believe the 35 U.S.C. §§103 and 112, second paragraph rejections have been overcome. Applicants respectfully request reconsideration of the Application and allowance thereof.

If the Examiner feels that a telephone conversation would expedite prosecution of this Application, he is asked to call Applicants' Agent at (617) 861-6240.

Respectfully submitted,

Richard W. Wagner

Richard W. Wagner
Agent for Applicants
Registration No. 34,480

Lexington, MA 02173

Dated: September 23, 1991

DOCKET NO. SPC89-C

365/215

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

RECEIVED
91 OCT -4 AM 9:35
GROUP 120

#10
JRP
10/5/91

Applicant(s): Timothy J. Barberich an James W. Young

Serial No.: 07/461,262

Group Art Unit: 125

Filed: January 5, 1990

Examiner: L. Schenkman

For: METHOD FOR TREATING ASTHMA USING OPTICALLY PURE R(-) ALBUTEROL

PETITION FOR EXTENSION OF TIME

The Honorable Commissioner
of Patents and Trademarks
Washington, D. C. 20231

Sir:

The undersigned attorney petitions the Commissioner of Patents and Trademarks to extend the time for filing a Response to the Office Action dated March 22, 1991 for 3 months from June 22, 1991 to September 22, 1991.

	Small Entity	Other than Small Entity
1 month -	___ $ 50	___ $ 100
2 months -	___ $ 150	___ $ 300
3 months -	X $ 365	___ $ 730
4 months -	___ $ 575	___ $ 1,150

[X] Enclosed is a check in the amount of $365.00 to cover the cost of the extension.

[ ] Please charge Deposit Account No. 08-0380 in the amount of $_____ to cover the cost of the extension fee.

Any deficiency or overpayment should be charged or credited to Deposit Account No. 08-0380. A duplicate copy of this letter is enclosed. 050 LP 10/03/91 07461262 1 217 365.00 CK

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to Honorable Commissioner of Patents and Trademarks, Washington, D.C. 20231, on 9/23/91.

Hamilton, Brook, Smith & Reynolds

B. J. Hennis
Signature

9/23/91
Date

Respectfully submitted,

Richard W. Wagner

Richard W. Wagner
Agent for Applicant(s)
Registration No. 34,480
Tel. (617) 861-6240
Lexington, MA 02173

Date: September 23, 1991

DLEV012086

SPC89-05 IDS
RWW2a
9/24/91
RWW/bjn

PATENT APPLICATION
Docket No. SPC89-05

#12

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Timothy J. Barberich and James W. Young

Serial No.: 07/461,262 Group Art Unit: 125

Filed: January 5, 1990 Examiner: L. Schenkman

Title: METHOD FOR TREATING ASTHMA USING OPTICALLY PURE R(-) ALBUTEROL

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to Honorable Commissioner of Patents and Trademarks, Washington, D.C. 20231 on 9/25/91

Hamilton, Brook, Smith & Reynolds, P.C.

B. J. Kennis 9/25/91
Signature Date

91 SEP 30 AM 10:56
GROUP 125

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT

The Honorable Commissioner
of Patents and Trademarks
Washington, D. C. 20231

Sir:

Pursuant to 37 C.F.R. 1.56 and 1.97-1.99, the following listed item is cited to the Examiner as being information which, in the good faith judgment of the Applicants and the undersigned Agent, is relevant to the subject matter claimed in the above-identified

Application. The following listed item has come to Applicants' attention since the Information Disclosure Statement of December 20, 1990 was mailed to the Patent Office.

As required by 37 C.F.R. 1.98, each item below is followed by a "concise explanation" of its possible relevance. The comments are an introduction intended to help the Examiner place each item in context. They are not represented or intended to be comprehensive summaries.

AV Tan et al., J. Chromatography 422, 187-95 (1987)

The authors describe measurements of the amounts of the R(-) and S(+) enantiomers of salbutamol in human urine following oral or intravenous administration of the racemic mixture. The studies show that the S(+) enantiomer is more rapidly excreted than the more active R(-) enantiomer by the renal route.

The reference is listed on the attached PTO Form 1449 and a copy is included for the Examiner's consideration.

Respectfully submitted,

Richard W. Wagner

Richard W. Wagner
Agent for Applicants
Registration No. 34,480
(617) 861-6240

Lexington, MA 02173

Dated: September 25, 1991

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
07/461,262	01/05/90	BARBERICH T	SPC8905

HAMILTON, BROOK, SMITH & REYNOLDS
TWO MILTIA DRIVE
LEXINGTON, MA 02173-4799

EXAMINER
SCHENKMAN, L

ART UNIT	PAPER NUMBER
1205	13

DATE MAILED: 12/09/91

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

☐ This application has been examined ☒ Responsive to communication filed on 9/26/91 ☐ This action is made final.

A shortened statutory period for response to this action is set to expire 3 month(s), ____ days from the date of this letter.
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

1. ☐ Notice of References Cited by Examiner, PTO-892.
2. ☐ Notice re Patent Drawing, PTO-948.
3. ☒ Notice of Art Cited by Applicant, PTO-1449.
4. ☐ Notice of Informal Patent Application, Form PTO-152
5. ☐ Information on How to Effect Drawing Changes, PTO-1474.
6. ☐ _____

Part II SUMMARY OF ACTION

1. ☒ Claims 1-6, 8, 9, 12 and 14 are pending in the application.
   Of the above, claims _____ are withdrawn from consideration.
2. ☐ Claims _____ have been cancelled.
3. ☐ Claims _____ are allowed.
4. ☒ Claims 1-6, 8, 9, 12 and 14 are rejected.
5. ☐ Claims _____ are objected to.
6. ☐ Claims _____ are subject to restriction or election requirement.
7. ☐ This application has been filed with informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.
8. ☐ Formal drawings are required in response to this Office action.
9. ☐ The corrected or substitute drawings have been received on _____. Under 37 C.F.R. 1.84 these drawings are ☐ acceptable; ☐ not acceptable (see explanation or Notice re Patent Drawing, PTO-948).
10. ☐ The proposed additional or substitute sheet(s) of drawings, filed on _____, has (have) been ☐ approved by the examiner; ☐ disapproved by the examiner (see explanation).
11. ☐ The proposed drawing correction, filed _____, has been ☐ approved; ☐ disapproved (see explanation).
12. ☐ Acknowledgement is made of the claim for priority under U.S.C. 119. The certified copy has ☐ been received ☐ not been received ☐ been filed in parent application, serial no. _____; filed on _____.
13. ☒ Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.
14. ☐ Other

PTOL-326 (Rev. 2-92) EXAMINER'S ACTION

The following is a quotation of 35 U.S.C. § 103 which forms the basis for all obviousness rejections set forth in this Office action:

> A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.
>
> Subject matter developed by another person, which qualifies as prior art only under subsection (f) or (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

Claims 1-6, 8, 9, 13 and 14 are rejected under 35 U.S.C. § 103 as being unpatentable over Chemical Abstracts for reasons of record. Applicant's arguments and analysis of the In re Adamson decision are not well taken. The fact that Adamson does not relate to treatment of asthma or use of the claimed isomer is not germane since the claimed isomer has the same type of activity as the racemic mixture.

Claims 1-5 are rejected under 35 U.S.C. § 103 as being unpatentable over Brittain et al., Hartley et al., Hawkins et al. and Buckner et al. for reasons of record. Applicant's argument that the prior art teaches that the (-) isomer and the racemic mixture exhibit the same degree of activity is not universally accepted; note that Hawkins et al. article. In any event, since

it has been established that the racemic mixture and isomeric forms of the compounds have been used or tested as bronchodilators in the treatment of asthma, the use of compositions containing the claimed isomer in the treatment of asthma is clearly rendered obvious, notwithstanding the inconsistency or the prior art on the this point. The references cited herein would present a strong prima facie case of obviousness even assuming, arguendo, they dealt solely with the racemic mixture.

Claims 6, 8, 9, 13 and 14 are rejected under 35 U.S.C. § 103 as being unpatentable over Brittain et al. Hartley et al. Hawkins et al. and Bruckner et al. in view of Chemical Abstracts for reasons of record. The fact that Chemical Abstracts does not teach the isomers of Albuterol is not germane to this rejection. Drug combinations containing the (-) isomer would clearly be obvious in view of the teaching of drug combinations containing the racemic mixture.

Claims 9, 13 and 14 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claims 13 and 14 do not have proper antecedent support in claim 9 which appears to be limited to the (-) isomer. Claims 9, 13 and 14 are again rejected as being too broad absent recitation of amounts of ingredients present. The

skilled artisan would be hard pressed to determine contemplated proportions; note the functional language of claim 6.

Tan et al. is cited to show the state of the art.

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 C.F.R. § 1.136(a).

A SHORTENED STATUTORY PERIOD FOR RESPONSE TO THIS FINAL ACTION IS SET TO EXPIRE THREE MONTHS FROM THE DATE OF THIS ACTION. IN THE EVENT A FIRST RESPONSE IS FILED WITHIN TWO MONTHS OF THE MAILING DATE OF THIS FINAL ACTION AND THE ADVISORY ACTION IS NOT MAILED UNTIL AFTER THE END OF THE THREE-MONTH SHORTENED STATUTORY PERIOD, THEN THE SHORTENED STATUTORY PERIOD WILL EXPIRE ON THE DATE THE ADVISORY ACTION IS MAILED, AND ANY EXTENSION FEE PURSUANT TO 37 C.F.R. § 1.136(a) WILL BE CALCULATED FROM THE MAILING DATE OF THE ADVISORY ACTION. IN NO EVENT WILL THE STATUTORY PERIOD FOR RESPONSE EXPIRE LATER THAN SIX MONTHS FROM THE DATE OF THIS FINAL ACTION.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Examiner Schenkman whose telephone number is (703) 308-4651.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-1235.

LEONARD SCHENKMAN
EXAMINER
ART UNIT 125

Schenkman: ach
December 05, 1991

Sheet 1 of 1

ATTY. DOCKET NO. SPC89-05

SERIAL NO. 08/463,581 07/461,262

17

APPLICANT Timothy J. Barberich and James W. Young

FILING DATE January 5, 1990

GROUP 125

U.S. PATENT DOCUMENTS

EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE IF APPROPRIATE

OTHER ART (Including Author, Title, Date, Pertinent Pages, Etc.)

AV	Tan et al., "Analysis of Salbutomol Enantiomers in Human Urine by Chiral High Performance Liquid Chromatography and Preliminary Studies Related to the Stereoselective Disposition Kinetics in Man", J. Chromatogr., 422, 187-95 (1987)

Examiner T. Schuchardt Date Considered 11-1991

DLEV012093

JUN 9 1992 U.S. PATENT AND TRADEMARK OFFICE

PATENT APPLICATION
Docket No. SPC 89-05

#14
JRP
6/22/92

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Timothy J. Barberich and James W. Young

Serial No: 07/461,262 Group Art Unit: 1205

Filed: January 5, 1990 Examiner: L. Schenkman

Title: METHOD FOR TREATING ASTHMA USING OPTICALLY PURE R(-) ALBUTEROL

"EXPRESS MAIL" Mailing Label No. RB 794623247
Date of Deposit: June 9, 1992
I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231
B. J. Naunis
(Typed or printed name of person mailing paper or fee)
B. J. Naunis
(Signature of person mailing paper or fee)

92 JUN 22 AM 10:26

ASSOCIATE POWER OF ATTORNEY

The Honorable Commissioner
of Patents and Trademarks
Washington, D. C. 20231

Sir:

An Associate Power of Attorney is hereby granted to Richard W. Wagner, Registration No. 34,480, in the above-identified patent application.

Please send all correspondence to Patricia Granahan, Hamilton, Brook, Smith & Reynolds, P.C., Two Militia Drive, Lexington, MA 02173. Please direct all telephone calls to Patricia Granahan at (617) 861-6240.

Respectfully submitted,

Patricia Granahan

Patricia Granahan
Registration No. 32,227
Attorney for Applicant(s)
(627) 861-6240

Lexington, MA 02173

Dated: June 9, 1992

DOCKET NO. SPC89-[redacted]

(SS/00) [redacted] .00 21.1 6F120

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

MAIL ROOM JUN 14 1992 PAT. & TRADE MARK OFF.

Applicant(s): Timothy J. Barberich and James W. Young

Serial No.: 07/461,262

Filed: January 5, 1990

Group Art Unit: 1205

Examiner: L. Schenkman

For: METHOD FOR TREATING ASTHMA USING OPTICALLY PURE R(-) ALBUTEROL

RB

"EXPRESS MAIL" Mailing Label No. 794623247
Date of Deposit June 9, 1992
I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231
B. J. Nannis
(Typed or printed name of person mailing paper or fee)
B. J. Nannis
(Signature of person mailing paper or fee)

PETITION FOR EXTENSION OF TIME

The Honorable Commissioner
of Patents and Trademarks
Washington, D. C. 20231

Sir:

The undersigned attorney petitions the Commissioner of Patents and Trademarks to extend the time for filing a Notice of Appeal to the Office Action Made Final dated December 9, 1991 for 3 months from March 9, 1992 to June 9, 1992
In lieu of a Notice of Appeal, Applicants are filing a File Wrapper Continuation application concurrently herewith.

	Small Entity	Other than Small Entity
1 month -	___$ 55	___$ 110
2 months -	___$175	___$ 350
3 months -	X $405	___$ 810
4 months -	___$640	___$1,280

/X/ Enclosed is a check in the amount of $ 405.00 to cover the cost of the extension.

/ / Please charge Deposit Account No. 08-0380 in the amount of $________ to cover the cost of the extension fee.

Any deficiency or overpayment should be charged or credited to Deposit Account No. 08-0380. Two duplicate copies of this letter are enclosed.

Respectfully submitted,
Richard W. Wagner
Richard W. Wagner
Agent for Applicant(s)
Registration No. 34,480
(617) 861-6240

Lexington, Massachusetts 02173

Dated: June 9, 1992

070 AA 06/17/92 07461262 1 217 405.00 CK

92 JUN 22 PM 4:00

92 JUN 22 PM 4:00

JUMBO

SERIAL NUMBER: 07 896725	PATENT DATE	PATENT NUMBER

SERIAL NUMBER	FILING DATE	CLASS	SUBCLASS	GROUP ART UNIT	EXAMINER
07/896,725	06/09/92	514	646	1205	Schenkman

APPLICANTS

TIMOTHY J. BARBARICH, CONCORD, MA; JAMES W. YOUNG, STILL RIVER, MA.

Henley

CONTINUING DATA********
VERIFIED THIS APPLN IS A CON OF 07/461,262 01/05/90
Schenkman

FOREIGN/PCT APPLICATIONS********
VERIFIED

FOREIGN FILING LICENSE GRANTED 06/30/92 ***** SMALL ENTITY *****

Foreign priority claimed 35 USC 119 conditions met	AS FILED	STATE OR COUNTRY	SHEETS DRWGS.	TOTAL CLAIMS	INDEP. CLAIMS	FILING FEE RECEIVED	ATTORNEY'S DOCKET NO.
Verified and Acknowledged / Examiner's Initials		MA	0	10	3	$345.00	SPC89-05

ADDRESS
PATRICIA GRANAHAN
HAMILTON, BROOK, SMITH & REYNOLDS
TWO MILITIA DRIVE
LEXINGTON, MA 02173

TITLE
METHOD FOR TREATING ASTHMA USING OPTICALLY PURE R(-) ALBUTEROL

U.S. DEPT. of COMM./Pat. & TM Office—PTO-436L (rev. 10-78)

PARTS OF APPLICATION FILED SEPARATELY

NOTICE OF ALLOWANCE MAILED	PREPARED FOR ISSUE		CLAIMS ALLOWED	
	Assistant Examiner	Docket Clerk	Total Claims	Print Claim
ISSUE FEE			DRAWING	
Amount Due / Date Paid		Primary Examiner	Sheets Drwg. / Figs. Drwg. / Print Fig.	
Label Area	ISSUE CLASSIFICATION: Class / Subclass		ISSUE BATCH NUMBER	

WARNING: The information disclosed herein may be restricted. Unauthorized disclosure may be prohibited by the United States Code Title 35, Sections 122, 181 and 368. Possession outside the U.S. Patent & Trademark Office is restricted to authorized employees and contractors only.

Form PTO-436A Rev. 9/90

BEST AVAILABLE COPY

POSITION	INIT.	DATE
CI ASSIGNED		

SEARCHED

Class	Sub.	Date	Exmr.
514	649		

INTERFERENCE SEARCHED

Class	Sub.	Date	Exmr.

SEARCH NOTES

	Date	Exmr.

BEST AVAILABLE COPY

SEARCHED

POSITION		INIT.	DATE
CLASSIFIER			
EXAMINER		414	6-26-92
TYPIST		342	6-30-92
VERIFIER		VRK	11/20/92
CORPS CORR.			
SPEC. HAND			
FILE MAINT.			

INDEX OF CLAIMS

Claim		Date
Final	Original	
	(1)	
	2	
	3	
	4	
	5	
	(6)	
	7	
	8	
	(9)	
	10	
	11	
	12	
	13	
	14	
	(15)	
	16	
	17	
	18	
	19	
	20	
	21	
	22	
	23	
	24	
	25	
	26	
	27	
	28	
	29	
	30	
	31	
	32	
	33	
	34	
	35	
	36	
	37	
	38	
	39	
	40	
	41	
	42	
	43	
	44	
	45	
	46	
	47	
	48	
	49	
	50	

Claim		Date
Final	Original	
	51	
	52	
	53	
	54	
	55	
	56	
	57	
	58	
	59	
	60	
	61	
	62	
	63	
	64	
	65	
	66	
	67	
	68	
	69	
	70	
	71	
	72	
	73	
	74	
	75	
	76	
	77	
	78	
	79	
	80	
	81	
	82	
	83	
	84	
	85	
	86	
	87	
	88	
	89	
	90	
	91	
	92	
	93	
	94	
	95	
	96	
	97	
	98	
	99	
	100	

BEST AVAILABLE COPY

MAIL ROOM JUN 9 1992 PAT. & TRADE MARK OFF.

-1-

07 896725

PATENT APPLICATION
DOCKET NO.: SPC89-05'

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Timothy J. Barberich and James W. Young

Prior Application Serial No.: 07/461,262 Attorney's Docket No.: SPC89-05'

Prior Application Filing Date: January 5, 1990

For METHOD FOR TREATING ASTHMA USING OPTICALLY PURE R(-) ALBUTEROL

Anticipated Classification of this Application:
Class_____ Subclass_____

Prior application:
Examiner: L. Schenkman
Art Unit: 1205

Commissioner of Patents and Trademarks
Box FWC
Washington, D.C. 20231

FILE WRAPPER CONTINUING APPLICATION (FWC)

1. This is a request for a filing under the file wrapper continuing application procedure, 37 CFR 1.62, for a

X continuation

___ divisional

___ continuation-in-part (for oath or declaration see II below)

___ attached is an amendment for added subject matter of prior application
Serial No. _____ filed on _____

CERTIFICATION UNDER 37 CFR 1.10

I hereby certify that this FWC transmittal and the documents referred to as attached therein are being deposited with the United States Postal Service on this date June 9, 1992 in an envelope as "Express Mail Post Office to Addressee" mailing Label Number RB 794623247 addressed to the:

Commissioner of Patents and Trademarks
Box FWC
Washington, D.C. 20231

Barbara J. Nagnis
(Type or print name of person mailing paper)

Barbara J. Nagnis
(Signature of person mailing paper)

(further particulars of prior application are)

1. Title (as originally filed) METHOD FOR TREATING ASTHMA USING OPTICALLY PURE R(-) ALBUTEROL
(and as last amended) _____

2. Name of applicant(s) (as originally filed and as last amended) and current correspondence address of applicant(s)

	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME
FULL NAME OF INVENTOR	Barbarich	Timothy	J.
	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
RESIDENCE & CITIZENSHIP	Concord	MA	U.S.A.
	POST OFFICE ADDRESS	CITY	STATE & ZIP CODE/ COUNTRY
POST OFFICE ADDRESS	73 Nashoba Road	Concord	MA 01742 U.S.A.
	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME
FULL NAME OF INVENTOR	Young	James	W.
	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
RESIDENCE & CITIZENSHIP	Still River	MA	U.S.A.
	POST OFFICE ADDRESS	CITY	STATE & ZIP CODE/ COUNTRY
POST OFFICE ADDRESS	295 Still River Road	Still River	MA 01467 U.S.A.
	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME
FULL NAME OF INVENTOR			
	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
RESIDENCE & CITIZENSHIP			
	POST OFFICE ADDRESS	CITY	STATE & ZIP CODE/ COUNTRY
POST OFFICE ADDRESS			

The above-identified application in which no payment of issue fee, abandonment of, or termination of proceedings has occurred, is hereby expressly abandoned as of the filing date of this new application. Please use all the contents of the prior application file wrapper, including the drawings, as the basic papers for the new application.

It is understood that secrecy under 35 U.S.C. 122 is hereby waived to the extent that if information or access is available to any one of the applications in the file wrapper of a 37 CFR 1.62 application, be it either this application or a prior application in the same file wrapper, the PTO may provide similar information or access to all the other applications in the same file wrapper.

II. Declaration or oath

A. Continuation or divisional

_X_ none required

B. Continuation-in-part

___ attached

___ an original

executed by (check all applicable items)

___ inventor(s).

___ legal representative of inventor(s) 37 CFR 1.42 or 1.43.

___ joint inventor or person showing a proprietary interest for inventor who refused to sign or cannot be reached. 37 CFR 1.47;

___ This is the petition required by 37 CFR 1.47 and the statement required by 37 CFR 1.47 is also attached. See item V below for fee.

___ not attached

Application is made by a person authorized under 37 CFR 1.41(c) on behalf of all of the above-named applicant(s). The declaration or oath, along with the surcharge required by 37 CFR 1.16(e), can be filed subsequently.

___ showing that the filing is authorized. (Not required unless called into question. 37 CFR 1.41(d).

III. ___ Please enter the previously filed amendment under 37 CFR 1.116 from the prior application.

IV. Fee Calculation

X The fees to be charged are to be based on the number of claims remaining as a result of the

___ attached preliminary amendment

___ the unentered amendment filed under 37 CFR 1.116 in the prior application

X the claims as on file in the prior application

CLAIMS FOR FEE CALCULATION

The filing fee has been calculated as shown below:

	(Col. 1) NO. FILED	(Col. 2) NO. EXTRA	SMALL ENTITY RATE	SMALL ENTITY FEE		OTHER THAN A SMALL ENTITY RATE	OTHER THAN A SMALL ENTITY FEE
FOR:							
BASIC FEE				$345	OR		$690
TOTAL CLAIMS	10 -20=	* 0	x10=	$ 0	OR	x20=	$
INDEP CLAIMS	3 -3=	* 0	x36=	$ 0	OR	x72=	$
[ ] MULTIPLE DEPENDENT CLAIM PRESENTED							
* If the difference in Col. 1 is less than zero, enter "0" in Col. 2			x110= Assignment Fee:	$	OR	x220= Assignment Fee:	$
			TOTAL:	$345.00		TOTAL:	

Small Entity Statement

___ A verified statement that this is a filing by a small entity is attached.

X The small entity statement was filed in the parent application Serial No. 07/461,262 on March 9, 1990 and its benefit under 37 CFR 1.28(a) is hereby claimed.

V. Fee Payment Being Made At This Time

Method of Payment of Fees

X attached is check in the amount of $ 345.00

___ charge Account No. _____ in the amount of $ _____

VI. Authorization to Charge Additional Fees

X The Commissioner is hereby authorized to charge payment of the following fees associated with this communication or credit any overpayment to Deposit Account No. 08-0380. Two duplicate copies of this letter are enclosed.

X Any additional filing fees required under 37 CFR 1.16.

X Any patent application processing fees under 37 CFR 1.17.

X The Commissioner is hereby authorized to charge payment of the following fees during pendency of this application or credit any overpayment to Deposit Account No. 08-0380. Two duplicate copies of this letter are enclosed.

X Any patent application processing fees under 37 CFR 1.17.

X Any filing fees under 37 CFR 1.16 for presentation of extra claims.

VII. Instructions as to Overpayment

X credit Account No. 08-0380

___ refund

VIII. Prior Foreign Applications and Claim to Priority - 35 U.S.C. 119

Listed below are foreign application(s) for patent or inventor's certificate on which priority is claimed. Also identified are any foreign application(s) for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

(Number)	(Country)	(Day/Month/Year filed)	Priority Claimed Yes	No
(Number)	(Country)	(Day/Month/Year filed)	Yes	No
(Number)	(Country)	(Day/Month/Year filed)	Yes	No

IX. Relate Back - 35 U.S.C. 120

X Amend the specification by inserting before the first line the sentence:

~~This~~ is a
X continuation
___ divisional
___ continuation-in-part

of co-pending application Serial No. 07/461,262 filed on January 5, 1990 now Abandoned

X. Assignment

X the prior application is assigned of record to Sepracor, Inc., 33 Locke Drive, Marlborough, MA 01752

___ an assignment of the invention to _____ is attached.

XI. Power of Attorney

The power of attorney in the prior application is to

Attorney	Registration No.
David E. Brook	22,592
James M. Smith	28,043
Leo R. Reynolds	20,884
Giulio A. DeConti, Jr.	31,503
Richard A. Wise	18,041
Patricia Granahan	32,227
Mary Lou Wakimura	31,804
Thomas O. Hoover	32,470
Paula A. Campbell	32,503
Alice C. Olek	33,542

a. X The power appears in the original papers in the prior application.
b. ___ The power does not appear in the original papers, but was filed on _____.
c. X A new* power has been executed and is attached.
d. X Address all future communications to:

Patricia Granahan
HAMILTON, BROOK, SMITH & REYNOLDS, P.C.
Two Militia Drive
Lexington, MA 02173

*Associate Power of Attorney

XII. Maintenance of Copendency of Prior Application

Please abandon the prior application when any petition for extension of time filed in that application is granted and when this application is granted a filing date so as to make this application copending with said prior application.

___ A petition, fee and response has been filed to extend the term for response in the pending prior application until _____.

X A petition and fee is attached to extend the term for response in the pending prior application until June 9, 1992.

___ The term for response in the pending prior application expires _____.

Richard W. Wagner
Type or print name of person signing

June 9, 1992
Date

Richard W. Wagner
Signature

P.O. Address of Signatory:
Two Militia Drive
Lexington, MA 02173

___ Inventor
___ Assignee of complete interest
___ Person authorized to sign on behalf of assignee
Title _____

X Attorney or agent of record
___ Filed under Rule 34(a)

Tel. No.: (617) 861-6240

Reg. No.: _____
(if applicable)

07 896725

PATENT APPLICATION SERIAL NO.

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

040 RP 06/23/92 07896725 1 201 345.00 CK SPCB9-05

PTO-1556
(5/87)

PATENT APPLICATION FEE DETERMINATION RECORD

Effective December 16, 1991

Application or Docket Number: 896725

CLAIMS AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE	SMALL ENTITY FEE	OR	OTHER THAN SMALL ENTITY RATE	OTHER THAN SMALL ENTITY FEE
BASIC FEE				$ 345.00	OR		$ 690.00
TOTAL CLAIMS	10 minus 20 =	*	x $10 =		OR	x $20 =	
INDEPENDENT CLAIMS	3 minus 3 =	*	x 36 =		OR	x 72 =	
MULTIPLE DEPENDENT CLAIM PRESENT			+ 110 =		OR	+ 220 =	
			TOTAL	345	OR	TOTAL	

* If the difference in column 1 is less than zero, enter "0" in column 2

CLAIMS AS AMENDED - PART II

AMENDMENT A	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE	SMALL ENTITY ADDITIONAL FEE	OR	OTHER THAN SMALL ENTITY RATE	OTHER THAN SMALL ENTITY ADDITIONAL FEE
Total	* 11	Minus	** 20	= —	x $10 =	—	OR	x $20 =	
Independent	* 3	Minus	*** 3	= —	x 36 =	—	OR	x 72 =	
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM					+ 110 =		OR	+ 220 =	
					TOTAL ADDIT. FEE	—	OR	TOTAL ADDIT. FEE	

AMENDMENT B	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE	SMALL ENTITY ADDITIONAL FEE	OR	OTHER THAN SMALL ENTITY RATE	OTHER THAN SMALL ENTITY ADDITIONAL FEE
Total	*	Minus	**	=	x $10 =		OR	x $20 =	
Independent	*	Minus	***	=	x 36 =		OR	x 72 =	
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM					+ 110 =		OR	+ 220 =	
					TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

AMENDMENT C	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE	SMALL ENTITY ADDITIONAL FEE	OR	OTHER THAN SMALL ENTITY RATE	OTHER THAN SMALL ENTITY ADDITIONAL FEE
Total	*	Minus	**	=	x $10 =		OR	x $20 =	
Independent	*	Minus	***	=	x 36 =		OR	x 72 =	
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM					+ 110 =		OR	+ 220 =	
					TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

Form PTO-875
(Rev. 12-91)

*U.S. Government Printing Office: 1992 — 308-850

Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

DLEV012107

DOCKET NO. SPC89-05'

12-X 7-22-92 #17

MAIL ROOM JUL 17 1992 PAT. & TRADEMARK OFFICE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Timothy J. Barberich and James W. Young

Serial No.: 07/896,725

Group Art Unit: 1205

Filed: June 9, 1992

Examiner: L. Schenkman

RECEIVED 92 JUL 22 AM 7:15 GROUP 120

For: METHOD FOR TREATING ASTHMA USING OPTICALLY PURE R(-) ALBUTEROL

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to Honorable Commissioner of Patents and Trademarks, Washington, D.C. 20231 on 7-14-92

Hamilton, Brook, Smith & Reynolds, P.C.

B.J. Kenyon 7-14-92
Signature Date

The Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

Transmitted herewith is a response in the above-identified application.

[X] Small entity status of this application under 37 C.F.R. 1.9 and 1.27 has been established by a verified statement previously submitted.

[ ] A verified statement to establish small entity status under 37 C.F.R. 1.9 and 1.27 is enclosed.

The fee has been calculated as shown below:

	(Col. 1) CLAIMS REMAINING AFTER AMENDMENT		(Col. 2) HIGHEST NO. PREVIOUSLY PAID FOR	(Col. 3) PRESENT EXTRA	SMALL ENTITY RATE	SMALL ENTITY ADDIT. FEE	OR	OTHER THAN SMALL ENTITY RATE	OTHER THAN SMALL ENTITY ADDIT. FEE
TOTAL	* 10	MINUS	** 10	= 0	x 10	$ 0		x 20	$
DEP.	* 3	MINUS	*** 3	= 0	x 36	$ 0		x 72	$
[ ] FIRST PRESENTATION OF MULTIPLE DEP. CLAIM					+110	$		+220	$
				TOTAL =		$ 0			$

☐ Please charge my Deposit Account No. 08-0380 in the amount of $_____.

☐ A check in the amount of $_____ is attached.

☐ A separate Petition for Extension of Time is being filed concurrently herewith.

☐ Payment for the extension fee is included with the petition.

☐ Deposit Account No. 08-0380 is being charged for the extension fee.

RECEIVED
92 JUL 22 AM 11:16
GROUP 120

☒ The Commissioner is hereby authorized to charge payment of the following fees associated with this communication or credit any overpayment to Deposit Account No. 08-0380.

☒ Any filing fees under 37 C.F.R. 1.16 for the presentation of extra claims.

☒ Any patent application processing fees under 37 C.F.R. 1.17.

Any extensions of time that are required to maintain this application in a pending status, if not included herewith, are hereby requested. The Commissioner is hereby authorized to charge such extension fees to Deposit Account No. 08-0380. Two copies of this transmittal letter are enclosed for accounting purposes.

Respectfully submitted,

HAMILTON, BROOK, SMITH & REYNOLDS

Richard W. Wagner

By Richard W. Wagner
Agent for Applicant(s)
Registration No. 34,480
(617) 861-6240

Dated: July 14, 1992

SPC89-05'Pre A
RWW12
7/14/92
RWW/bjn

PATENT APPLICATION
Docket No. SPC89-05

#17/A
JK
7/22/92

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Timothy J. Barberich and James W. Young

Serial No.: 07/896,725 Group Art Unit: 1205

Filed: June 9, 1992 Examiner: L. Schenkman

Title: METHOD FOR TREATING ASTHMA USING OPTICALLY PURE R(-) ALBUTEROL

RECEIVED
92 JUL 22 AM 7:16
GROUP 120

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to Honorable Commissioner of Patents and Trademarks, Washington, D.C. 20231 on 7-14-92

Hamilton, Brook, Smith & Reynolds, P.C.

B.J. Kenny 7-14-92
Signature Date

PRELIMINARY AMENDMENT

The Honorable Commissioner
of Patents and Trademarks
Washington, D. C. 20231

Sir:

Please amend the above-identified Application as follows:

In the Claims:

In Claim 1, line 6, between "bronchodilation" and the "," insert ---while simultaneously reducing undesirable side effects---;

In Claim 6, line 6, between "bronchodilation" and "and" insert ---while simultaneously reducing undesirable side effects---;

D2

In Claim 9, line 2, delete "an optically pure" and instead insert ---the---.

REMARKS

The instant Application is a contination of Application Serial No. 07/461,262 ("the parent case").

The above amendments to the Claims have been made to more distinctly claim the subject matter of the invention. Support for these amendments can be found on page 2, line 6-page 3, line 6; page 3, lines 8-14; and page 6, lines 14-27 of the specification. The relationship between these amendments to the Claims and the response to the Office Action of December 9, 1991 in the parent case will be more fully explained below.

Rejection of Claims 1-6, 8, 9, 13 and 14 under 35 U.S.C. §103.

Claims 1-6, 8, 9, 13 and 14 were rejected under 35 U.S.C. §103 over Chemical Abstracts which, as previously stated by the Examiner, teaches salbutamol (albuterol) used to treat asthma and compositions containing albuterol. The case, In re Adamson et al., was cited as teaching that the difference in activity between isomers is not unexpected.

Applicants respectfully submit that the claims, as amended, overcome the rejection. The Chemical Abstracts reference shows the bronchodilation effects of salbutamol and drug combinations incorporating salbutamol. However, this reference does not teach the use of a quantity of the R(-) isomer of albuterol sufficient to cause bronchodilation while simultaneously reducing undesirable side effects associated with racemic albuterol.

Although In re Adamson et al. teaches that optical isomers themselves are unpatentable over compounds that the art recognizes as having optical isomers, it is not correct to assume from this that a new method for using an isomer is unpatentable, particularly where, as here, the method unexpectedly provides therapeutic effects without causing undesirable side effects.

One would be led to assume, from the Examiner's apparent interpretation of In re Adamson et al., that the physiological effects of a racemic compound, both therapeutic and adverse, are elicited by the same isomer. However, this assumption is contrary to Applicants' disclosure which teaches that undesirable side effects are associated with the racemic mixture or the therapeutically inactive isomer, i.e. the S(+) isomer, of albuterol, but not with the R(-) isomer. Applicants have, therefore, made the unexpected disclosure that the claimed isomer does not have the same type of activity as the racemic mixture.

Rejection of Claims 1-5 under 35 U.S.C. §103.

Claims 1-5 have been rejected under 35 U.S.C. §103 as being unpatentable over Brittain et al., Hartley et al., Hawkins et al., and Buckner et al. who, as previously stated by the Examiner, teach compositions containing the claimed compounds with its isomers used as a bronchodilator in the treatment of asthma and, further, that the R(-) isomer has greater bronchodilation activity over the S(+) isomer.

Applicants respectfully submit that the Claims, as amended, also overcome this rejection. In addition to a complete lack of agreement among the cited references concerning the relative efficacies of the R(-) isomer and the racemate, there is no teaching in these references regarding the administration of a quantity of the R(-) isomer sufficient to effect bronchodilation but without

causing undesirable side effects. The references do not indicate that undesirable side effects can be minimized by administering one of the isomers. Only Applicants' disclosure reveals and claims this important method by administering the R(-) isomer of albuterol.

Rejection of Claims 6, 8, 9, 13 and 14 under 35 U.S.C. §103.

Claims 6, 8, 9, 13 and 14 have been rejected under 35 U.S.C. §103 as being unpatentable over Brittain et al., Hartley et al., Hawkins et al. and Buckner et al. in view of Chemical Abstracts which, as previously stated by the Examiner, shows combinations of drugs, including salbutamol, used in the treatment of asthma.

Applicants respectfully traverse this rejection, particularly as applied to the presently amended claims. Although drug combinations including racemic salbutamol are shown in Chemical Abstracts, there is no indication that a combination containing the R(-) isomer minimizes the undesirable side effects associated with the racemic mixture of albuterol. The combination of the other cited references also does not show this element. The combination of drugs which includes the R(-) isomer would not be obvious since undesirable side effects would be expected to be associated with it; there would be no benefit associated with using the R(-) isomer compared with using the racemic mixture. However, Applicants' disclosure shows that undesirable side effects are minimized when the R(-) isomers used. Thus, the combination of drugs including R(-) albuterol is not an obvious extension of a combination of drugs including racemic albuterol.

Rejection of Claims 9, 13 and 14 under 35 U.S.C. §112, second paragraph.

Claims 9, 13 and 14 have been rejected under 35 U.S.C. §112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter of the invention. It was stated that Claims 13 and 14 do not have proper antecedent support in Claim 9. Claims 9, 13 and 14 were also rejected as being too broad absent recitation of amounts of ingredients present.

Claim 9 has been presently amended to remove the phrase "optically pure". It is believed that Claims 13 and 14 now have proper antecedent basis and specify the amount of purity of the R(-) isomer of albuterol.

Applicants again respectfully traverse the rejection of Claims 9, 13 and 14 because recitations of amounts of ingredients are dependent on a number of physiological factors which make specification of quantities uncertain until the physiological features are known. It is submitted that skilled artisans, when these features are known, can determine the amounts of ingredients based on these physiological factors.

CONCLUSIONS

With the above amendments and for the above stated reasons, Applicants believe the 35 U.S.C. §§103 and 112, second paragraph rejections have been overcome. Applicants respectfully request reconsideration of the Application and allowance thereof.

If the Examiner feels that a telephone conversation would expedite the prosecution of this Application, he is asked to call Applicant's Agent at (617) 861-6240.

Respectfully submitted,

Richard W. Wagner

Richard W. Wagner
Registration No. 34,480
Agent for Applicant

Lexington, MA 02173
Dated: July 14, 1992

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
07/[illegible],726	06/03/92	[illegible]	SP089-05

EXAMINER
ST.RENYHAN,L

ART UNIT: 1205 | PAPER NUMBER: 18

DATE MAILED: 08/18/92

PATRICIA STANTON
HAMILTON, BROOK, SMITH & REYNOLDS
530 MILITIA DRIVE
LEXINGTON, MA 02173

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

☑ This application has been examined ☐ Responsive to communication filed on _____ ☐ This action is made final.

A shortened statutory period for response to this action is set to expire 3 month(s), ___ days from the date of this letter.
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

1. ☐ Notice of References Cited by Examiner, PTO-892.
2. ☐ Notice re Patent Drawing, PTO-948.
3. ☐ Notice of Art Cited by Applicant, PTO-1449.
4. ☐ Notice of Informal Patent Application, Form PTO-152.
5. ☐ Information on How to Effect Drawing Changes, PTO-1474.
6. ☐ _____

Part II SUMMARY OF ACTION

1. ☑ Claims 1-6, 8, 9, 13, 14 are pending in the application.
   Of the above, claims _____ are withdrawn from consideration.
2. ☐ Claims _____ have been cancelled.
3. ☐ Claims _____ are allowed.
4. ☑ Claims 1-4, 8, 9, 13, 14 are rejected.
5. ☐ Claims _____ are objected to.
6. ☐ Claims _____ are subject to restriction or election requirement.
7. ☐ This application has been filed with informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.
8. ☐ Formal drawings are required in response to this Office action.
9. ☐ The corrected or substitute drawings have been received on _____. Under 37 C.F.R. 1.84 these drawings are ☐ acceptable. ☐ not acceptable (see explanation or Notice re Patent Drawing, PTO-948).
10. ☐ The proposed additional or substitute sheet(s) of drawings, filed on _____ has (have) been ☐ approved by the examiner. ☐ disapproved by the examiner (see explanation).
11. ☐ The proposed drawing correction, filed on _____, has been ☐ approved. ☐ disapproved (see explanation).
12. ☐ Acknowledgment is made of the claim for priority under U.S.C. 119. The certified copy has ☐ been received ☐ not been received
    ☐ been filed in parent application, serial no. _____; filed on _____
13. ☐ Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.
14. ☐ Other

PTOL-326 (Rev. 9-89) EXAMINER'S ACTION

The following is a quotation of 35 U.S.C. § 103 which forms the basis for all obviousness rejections set forth in this Office action:

> A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.
>
> Subject matter developed by another person, which qualifies as prior art only under subsection (f) or (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

Claims 1-6, 8, 9, 13 and 14 are rejected under 35 U.S.C. § 103 as being unpatentable over Chemical Abstracts which teaches salbutamol (albuterol) used to treat asthma and compositions containing same. The determination of a particular isomer to employ would be a matter of obvious alternative to one skilled in the art.

Claims 1-5 rejected under 35 U.S.C. § 103 as being unpatentable over Brittain et al, Hartley et al, Hawkins et al and Buckner et al. for reasons of record as set forth in Paper no. 9 (Office action dated 3/22/92).

Claims 6, 8, 9, 13 and 14 rejected under 35 U.S.C. § 103 as being unpatentable over Brittain et al, Hartley et al, Hawkins et al and Buckner et al in view of Chemical Abstracts for reasons of

record as set forth in Paper no. 9.

Applicants' remarks regarding the prior art rejections supra are not persuasive. The arguments set forth therein have been previously considered and commented upon in the parent application.

Claims 9, 13 and 14 rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claims 13 and 14 do not have proper antecedent support in claim 9. Amended claim 9 still refers exclusively to the R(-) isomer. Claims 9, 13 and 14 are too broad absent recitation of amounts of ingredients present. Applicant's comments that they are not sure what the compositions would be used for is not persuasive.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Examiner Schenkman whose telephone number is (703) 308-4651.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-1235.

LEONARD SCHENKMAN
EXAMINER
ART UNIT 125

SCHENKMAN:te
August 04, 1992

MAIL ROOM FEB 16 1993 PAT. & TRADEMARK OFF.

PATENT APPLICATION
DOCKET NO. SPC89-05'

#23

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Timothy J. Barberich and James W. Young

Serial No.: 07/896,725 Group Art Unit: 1205

Filed: June 9, 1992 Examiner: L. Schenkman

For: METHOD FOR TREATING ASTHMA USING OPTICALLY PURE R(-) ALBUTEROL

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to Honorable Commissioner of Patents and Trademarks, Washington, D.C.20231, on

February 10, 1993

B.J. Mannia
Signature

February 10, 1993
Date

The Honorable Commissioner
of Patents and Trademarks
Washington, D.C. 20231

Sir:

Transmitted herewith is a response in the above-identified application.

[X] Small entity status of this application under 37 C.F.R. 1.9 and 1.27 has been established by a verified statement previously submitted.

[ ] A verified statement to establish small entity status under 37 C.F.R. 1.9 and 1.27 is enclosed.

The fee has been calculated as shown below:

	(COL. 1) CLAIMS REMAINING AFTER AMENDMENT		(COL. 2) HIGHEST NO. PREVIOUSLY PAID FOR	(COL. 3) PRESENT EXTRA
TOTAL	* 11	MINUS	** 21	0
INDEP	* 3	MINUS	*** 3	0
[ ] FIRST PRESENTATION OF MULTIPLE DEP. CLAIM				

SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
RATE	ADDIT. FEE		RATE	ADDIT. FEE
X 11	$ 0		X 22	$
X 37	$ 0		X 74	$
+115	$		+230	$
TOTAL =	$ 0			$

DLEV012119

[ ] Please charge my Deposit Account No. 08-0380 in the amount of $_____.

[ ] A check in the amount of $_____ is attached.

[X] A separate Petition for Extension of Time is being filed concurrently herewith.

[X] Payment for the extension fee is included with the petition.

[ ] Deposit Account No. 08-0380 is being charged for the extension fee.

[X] The Commissioner is hereby authorized to charge payment of the following fees associated with this communication or credit any overpayment to Deposit Account No. 08-0380.

[X] Any filing fees under 37 C.F.R. 1.16 for the presentation of extra claims.

[X] Any patent application processing fees under 37 C.F.R. 1.17.

Any extensions of time that are required to maintain this application in a pending status, if not included herewith, are hereby requested. The Commissioner is hereby authorized to charge such extension fees to Deposit Account No. 08-0380. Two copies of this transmittal letter are enclosed for accounting purposes.

Respectfully submitted,

HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

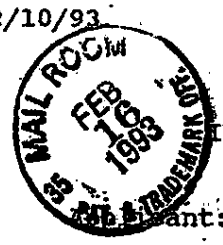
By Richard W. Wagner

Richard W. Wagner
Registration No. 34,480
Agent for Applicant(s)
(617) 861-6240

Dated: Feb. 10, 1993

A:\AMFEE.FOR

SPC89-05'
RWW13
2/10/93



PATENT APPLICATION
Docket No. SPC89-05'

#23/2
JR
3/18/93

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Timothy J. Barberich and James W. Young

Serial No.: 07/896,725 Group Art Unit: 1205

Filed: June 9, 1992 Examiner: L. Schenkman

Title: METHOD FOR TREATING ASTHMA USING OPTICALLY PURE R(-) ALBUTEROL

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to Honorable Commissioner of Patents and Trademarks, Washington, D.C. 20231 on 2/10/93

Hamilton, Brook, Smith & Reynolds, P.C.

B. J. Dennis 2/10/93
Signature Date

AMENDMENT C

The Honorable Commissioner
of Patents and Trademarks

Washington, D.C. 20231

Dear Sir:

This is in response to the official action of August 10, 1992, which in view of the petition for a three month extension of time submitted herewith, requires response by February 10, 1993.

Please amend the application as follows:

In the Claims:

Please cancel claims 9, 13 and 14 and substitute therefor new claims 15, 16, 17 and 18.

15. A pharmaceutical composition comprising:
(a) a first component consisting of an antiasthmatically effective amount of albuterol, said albuterol consisting of about 90 to 100% by weight of its R(-) isomer; and
(b) a second component consisting of a physiologically effective amount of a drug selected from the group consisting of bronchodilators, antihistamines and analgesics.

16. A composition according to claim 15 wherein said second component is an antiasthmatically effective amount of theophylline or terbutaline.

17. A composition according to claim 15 wherein said second component is an analgesically effective amount of a drug selected from the group consisting of aspirin, acetaminophen and ibuprofen.

18. A composition according to claim 15 wherein said albuterol is greater than 99% by weight R(-)-albuterol.

Remarks

The claims have been amended to include the amount (in functional terms) of the components to be included and to clarify the proportion of albuterol that is present as its R-isomer. Support for claim 16 is found on page 5, line 14; support for claim 17 is found on page 5, line 15 to line 16. Claim 18 replaces former claim 14 and makes it properly dependent on newly introduced claim 15.

Claims 1 to 6, 8, 9, 13 and 14 were presented in the application as filed. Claims 9, 13 and 14 have been cancelled and claims 15 through 18 have been added. Claims 1 to 6, 8 and 15 to 18 are therefore presently pending in the application.

Claims 1 to 6 and 8 stand rejected under 35 U.S.C. 103 as obvious over Chemical Abstracts. Claims 1 to 5 stand further rejected under 35 U.S.C. 103 as unpatentable over Brittain et al., Hartley et al., Hawkins et al. and Buckner et al. Claims 6 and 8 stand further rejected under 35 U.S.C. 103 as unpatentable over the latter four references in view of Chemical Abstracts. These rejections are traversed, and reconsideration is requested, for the following reasons:

The thrust of applicants' invention is the treatment of asthma while reducing the side effects associated with the administration of racemic albuterol. Side effects of drugs which, like albuterol, have a predominant β_2 agonist component, can arise from four presently recognized interactions, as discussed in the declaration under 37 C.F.R. 1.132 by Dr. Gunnar Aberg submitted herewith and rephrased below:

(a) non-adrenergic effects (there is no evidence for this among the references cited in the present case);

(b) interaction of the β-agonist with α receptors; (Second generation β-agonists like albuterol are relatively free of this problem.)

(c) interaction of the primarily β_2-agonist drug with β_1 receptors; and

(d) interaction of β_2-agonists with β_2 receptors giving rise to tachyphylaxis and perhaps to sensitization and CNS effects such as excitement and hyperkinesia.

Tachyphylaxis in response to albuterol has been demonstrated in airways [See Passowicz Muszynska Index Medicus Abstr. 91164287 (1991) (Attachment A); and Pauwels Index Medicus Abstr. 86051970 (1986)] (Attachment B). Sensitization has likewise been reported [See Chapman et al. Brit. J. Pharmacol. 99, 66P (1990)] (Attachment C). The mechanisms of these side effects are not clear and may not be the same.

The Brittain, Hartley, Hawkins and Buckner references all address the comparative interaction of albuterol isomers with β_1

vs β_2 receptors, a type (c) interaction according to the definition above. Three of these references show that there is perhaps some slight potency advantage to the use of pure R(-) albuterol vs. racemic albuterol (although Hartley shows a potency advantage to racemic albuterol), but none shows that there is any β-selectivity advantage to R over S or over racemic. On the contrary, Buckner concluded that the ratios of tracheal (β_2) to atrial (β_1) activities of R and S are indistinguishable. Side effects that are based on type (c) interactions arise from differences in receptor selectivity, and the person of ordinary skill would conclude from the teachings of these four references that there is no advantage of R over racemic in terms of expected amelioration of side effects. The Aberg Declaration establishes that the references by Brittain, Hartley, Hawkins and Buckner do not teach any expectation of decreased side effects from the administration of the pure R isomer as compared to the racemate.

Thus, at the time of filing of applicants' parent application (1/5/90), there were no teachings among the references cited that would motivate a person of ordinary skill to administer the pure R(-) isomer of albuterol for the treatment of asthma on the basis of its receptor selectivity.

What about potency? Even though applicants' disclosure does not relate to potency, does the art nonetheless encourage the person of ordinary skill to resolve and administer pure R albuterol on the basis of potency? Unless one pure enantiomer antagonizes the effects of the other, the theoretical advantage of a pure enantiomer is at most two-fold. A racemate, being a 50:50 mixture, simply acts like half a dose of the pure enantiomer and half a dose of filler. Because chemical resolution of racemic mixtures is never 100% efficient, a resolution will always yield less than 50% of the single isomer. Thus, unless one enantiomer antagonizes the effect of the other, there is no reason to suffer the loss of material attendant upon their resolution. For example, it has been known for years that

the activity of metoprolol as a β- blocker resides in its S isomer, but no one has ever marketed pure S-metoprolol because there has been no motivation to go to the trouble of removing the R isomer.

A potency ratio significantly greater than 2 between a single enantiomer and its racemate would be consistent with antagonism by one enantiomer and would provide motivation for resolving the racemate. No such teaching is found in any of the references. Choosing the single most optimistic experimental result from among the results of three tissues in only one of the four references, one may derive a 2.3 fold potency ratio for a single (R) isomer vs racemate. This falls in the range described above for "active isomer plus filler" and provides no motivation to undertake a separation of isomers. And these are the most encouraging data selected by hindsight reconstruction; the rest of the references, taken together, fairly suggest no clear preference of one isomer. Therefore, at the time of filing, the art did not suggest using pure R(-) albuterol either for lessened side effects or for potency enhancement. This conclusion is supported by the Declaration of Dr. Aberg. (The articles referred to by Dr. Aberg which have not been previously cited in this Application are included with the Declaration of Dr. Aberg as Exhibits 1, 2 and 3.)

Applicants disclose an unexpected diminution in side effects when the pure R isomer is administered. In support of this, applicants now cite two publications by the group of Morley and Chapman which appeared subsequent to the filing of the application: Morley, Chapman et al. Brit. J. Parmacol. 104 Suppl, 295P (1991) and Chapman et al. Trends in Pharmacol. Sci. 13 231-232 (1992). The significance of their disclosures is discussed in the Declaration by Dr. Aberg and copies are enclosed for the convenience of the Examiner as Exhibits 2 and 3. In these papers, the first of which was presented at a conference in September 1991, Morley et al. address the question of a distinction between a single enantiomer and racemic albuterol in

a type (d) interaction, thus supporting the concept of lessened side effects by the administration of pure R isomer.

The Morley and Chapman references disclose that the S(+) isomer in bronchial tissue causes a hypersensitivity to allergen. The authors conclude from their experiments that the desired bronchodilator effect (due to the R isomer) is prone to tachyphylaxis, while the undesired hypersensitivity (due to the S isomer) is less prone to tachyphylaxis. The authors state "It has long been recognized that use of sympathomimetics for asthma therapy is associated with a range of inconsistent or frankly paradoxical effects....our findings indicate that it may be prudent to remove enantiomers that were previously thought to be biologically inert." (Chapman et al. p. 232) Thus, the use of the pure R isomer is concluded to provide unexpected advantages. Applicants' disclosure of removing the S isomer so as to reduce side effects, and claims directed thereto, dating to at least January 1990 are novel and nonobvious -- particularly as evidenced by the subsequent Morley and Chapman publications.

For the foregoing reasons the rejections of claims 1-6 and 8 under 35 U.S.C. 103 are believed overcome. Reconsideration and withdrawal of the rejections are requested.

Claims 9, 13 and 14 which had been rejected under 35 U.S.C. 112 are now cancelled. Claim 15, which replaces claim 9, now clarifies that the pharmaceutical composition comprises from 90 to 100% of the R isomer. The Examiner had also asserted that former claims 9, 13 and 14 were too broad, absent recitation of amounts of ingredients. The claims have been amended to incorporate in functional terms the amounts of the ingredients. That such functional language is definite, allowable and common practice in the pharmaceutical art is illustrated in U.S. patents 4,975,426, claim 1, 4,923,898, claim 1 and 5,025,019, claim 1, copies of which are included for the convenience of the Examiner as attachments D, E and F, respectively. The rejections under 35 U.S.C. 112 are therefore believed overcome, and reconsideration and withdrawal is requested.

There being no further issues the application is believed in condition for allowance and such is requested.

Respectfully submitted,

Richard W. Wagner

Richard W. Wagner
Agent for Applicants
Registration No. 34,480

Lexington, MA 02173

Dated: February 10, 1993

F

3/5/4
7645287 91164287
[Effect on beta adrenergic receptors of tachyphylaxis on the sensitivity f smooth muscle in the bronchi to beta adrenergic receptor agonists in ronchial asthma]
WpLyw tachyfilaksji beta-adrenergicznych receptorow na wra.ANG.zliwosc iesni gLadkich oskrzeli na agoniste receptorow beta-adrenergicznych w ychawicy oskrzelowej.
Passowicz-Muszynska E
Katedry i Kliniki Chorob Wewnetrznych AM we WrocLawiu.
Pol Tyg Lek Jul 16-30 1990, 45 (29-31) p608-11, ISSN 0032-3756
ournal Code: PBY
Languages: POLISH Summary Languages: ENGLISH
Document type: JOURNAL ARTICLE English Abstract
RNAL ANNOUNCEMENT: 9106
S...file: INDEX MEDICUS
The study involved 30 subjects: 15 healthy individuals and 15 patients ith atopic bronchial asthma of the moderate degree. Salbutamol was dministered to asthmatic patients in the intravenous infusion for 7 days. eta-adrenergic receptor density in the lymphocytes and FEV1 were evaluated efore and after therapy. Moreover, isoprenaline test was carried out to valuate the sensitivity of the bronchial smooth muscle to beta-agonist. he test was performed prior to and after salbutamol therapy. It was found hat beta-receptor agonist statistically significantly decreases eta-adrenergic receptor density. Equivalently, bronchial smooth muscle is ess sensitive to beta-agonist in the same degree as a decrease in eta-adrenergic receptor density in the peripheral blood lymphocytes.
Tags: Female; Human; Male
Descriptors: *Albuterol--Therapeutic Use--TU; *Asthma--Drug Therapy--DT; Bronchi--Drug Effects--DE; *Muscle, Smooth--Drug Effects--DE; *Receptors, drenergic, Beta--Drug Effects--DE; *Tachyphylaxis--Physiology--PH; dolescence; Adult; Asthma--Physiopathology--PP; Lymphocytes--Drug Effects --DE
CAS Registry No.: 0 (Receptors, Adrenergic, Beta); 18559-94-9 (Albuterol)

[redacted]

ATTACHMENT A

G

3/5/18
05750970 86051970
[Effect of corticosteroids on the action of sympathomimetics]
Influence des corticosteroides sur l'action des sympathicomimetiques.
Pauwels R
Bull Eur Physiopathol Respir Sep-Oct 1985, 21 (5) p53s-55s, ISSN 0395-3890 Journal Code: BGX
Languages: FRENCH Summary Languages: ENGLISH
Document type: JOURNAL ARTICLE; REVIEW English Abstract
JOURNAL ANNOUNCEMENT: 8603
Subfile: INDEX MEDICUS
Corticosteroids restore the bronchial responsiveness to beta-adrenergic

stimulants in man. This has been shown both in severe asthmatic patients and in normal subjects, rendered insensitive by artificial means. On the contrary, in patients with bronchial asthma who have airways reactive to beta-adrenergic stimulants, the combination of corticosteroids and sympathicomimetics results in an additive effect of their bronchodilating capacity. Animal models, both in vivo and in vitro, show the same type of interaction between corticosteroids and beta-adrenergic stimulants. The mechanism by which corticosteroids restore the bronchial sensitivity to beta-adrenergic stimulation is not completely understood. Several mechanisms may be involved such as increased agonist binding, decreased receptor turn-over, increased uncoupling between receptor and adenylcyclase, decreased extraneuronal uptake, decreased COMT-activity. The relevance of the influence of corticosteroids on the metabolism of membrane phospholipids remains highly speculative. (15 Refs.)
Tags: Human
Descriptors: *Adrenal Cortex Hormones--Therapeutic Use--TU; *Adrenergic Beta Receptor Agonists--Therapeutic Use--TU; *Asthma--Drug Therapy--DT; Albuterol--Therapeutic Use--TU; Bronchodilator Agents--Therapeutic Use--TU; Drug Synergism; Drug Tolerance; Hydrocortisone--Therapeutic Use--TU; Isoproterenol--Therapeutic Use--TU; Methylprednisolone--Therapeutic Use--TU ; Prednisolone--Therapeutic Use--TU; Pregnenediones--Therapeutic Use--TU; Tachyphylaxis; Terbutaline--Therapeutic Use--TU
CAS Registry No.: 0 (Adrenal Cortex Hormones); 18559-94-9 (Albuterol) ; 23031-25-6 (Terbutaline); 50-23-7 (Hydrocortisone); 50-24-8 (Prednisolone); 51333-22-3 (budesonide); 7683-59-2 (Isoproterenol); 83-43-2 (Methylprednisolone)

ATTACHMENT B

GUINEA-PIGS, BUT SUPPRESSES RESPONSES TO FMLP

A. Imaizumi, J. Lefort & B.B. Vargaftig. Unité de Pharmacologie Cellulaire, Unité Associée Institut Pasteur-INSERM n° 285, 25 rue du Dr Roux, 75015, Paris, France.

Mice and rats inoculated with *Bordetella pertussis* vaccine show increased sensitivity to histamine, serotonin and anaphylaxis (Parfentjev and Goodline, 1948; Kind, 1958). This has been attributed to an acquired imbalance of two adrenergic effector systems, i.e., to a reduced functioning of the β-adrenergic receptors or of some of the reactions between receptor activation and adrenergic end-response (Szentivanyi, 1968). We have shown that enhanced bronchoconstriction, BC (i.e., unspecific broncho-pulmonary hyperresponsiveness) follows the administration of a booster injection of antigen to actively sensitized guinea-pigs (Pretolani et al., 1988). This led us now to study the effects of pertussis toxin (PT), the active component of *B. pertussis* on broncho-pulmonary responsiveness. PT was administered i.v. to guinea-pigs at 0.8-20 μg/kg 6-72 h before they were stimulated, under pentobarbitone anesthesia, with i.v. histamine (0.5-18 μg/kg) or serotonin (0.5-8 μg/kg), at 10 min intervals. Bronchial resistance to inflation was evaluated by the method of Konzett-Rössler in cm H_2O. PT induced leukocytosis (lymphocytosis), and in 10 animals the number of circulating leukocytes increased from 5,700±800 to 38,900±3,700 at the dose of 20 μg/kg after 72 h. This effect was dose and time-dependent and started within 6 h. Initially no differences were observed between the bronchoconstrictor responses to histamine or to serotonin of control and PT-treated animals but, when propranolol was used (1 mg/kg i.v. and 3 mg/kg i.p.), BC was slightly increased only (% BC: 13.4±2.8 up to 19.6±3.5) in control, but was markedly increased (% BC: 8.9±2.8 to 70.5±4.4, p<0.001) in animals treated 72 h beforehand with PT at 20 μg/kg. Similar effects were observed with serotonin. In contrast, BC and the accompanying leukopenia induced by the i.v. administration of the secretagogue *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP) (Boukili et al., 1986 and 1989) were antagonized by PT. Because of the contrasting effects on fMLP and on histamine and serotonin, isolated lungs provided by PT-treated animals where used . Under those conditions, BC and histamine and thromboxane A2 releases induced by the intra-pulmonary administration of fMLP were suppressed but the effects of OA (3 ng-100 μg injected to the lungs of guinea-pigs immunized with 10 μg ovalbumin (OA) in $Al(OH)_3$ injected i.p. twice, at a 2-week interval) were enhanced. PT thus modifies negatively the signal transductions for cells involved in the lung responses to fMLP, but positively the effects of the direct constrictor agents histamine and serotonin and of antigen, which induces BC via these mediators. Our data suggest that PT prevents the effects of fMLP on a target other than the neutrophil, since it was effective on the isolated lungs (Boukili et al., 1989), possibly via its recognized effects on the Gi protein of other effector systems present in the lung. Hyperresponsiveness may result from an enhanced mediator release, possibly due to down regulation of a Gi protein, associated to a direct effect on smooth muscle, at a level which is under investigation.

1. Boukili, M.A., Bureau, M., Lagente, V., Lefort, J., Lellouch-Tubiana, A., Malanchère, E. & Vargaftig, B.B. (1986) Br. J. Pharmac., 89, 349-359.
2. Boukili, M.A. Bureau, M., Lellouch-Tubiana, A., Lefort, J., Simon, M. & Vargaftig, B.B. (1989) Br. J. Pharmac., 98, 61-70.
3. Kind, L.S. (1958) Bact. Rev., 22, 173.
4. Parfentjev, I.A. & Goodline, M.A. (1948) J. Pharmac. exp. Ther., 92, 411.
5. Pretolani, M., Lefort, J. & Vargaftig B.B. (1988) Am. Rev. Respir. Dis., 138, 1572-1578.
6. Szentivanyi, A. (1968) J. Allergy, 42, 203-232.

66P AN ANOMALOUS EFFECT OF SALBUTAMOL IN SENSITISED GUINEA-PIGS

I. Chapman*, L. Mazzoni & J. Morley, Preclinical Research, Sandoz Ltd., Basel CH-4002, Switzerland.

Eosinophils migrate to the intrapulmonary airways of sensitised guinea-pigs in response to inhaled allergen. Whilst assessing the capacity of anti-asthma drugs to inhibit this phenomenon, it was noted that animals pretreated with salbutamol (S) (1 mg/kg/day) by subcutaneous infusion invariably died on inhalation of allergen, in marked contrast to animals that were untreated or received other anti-asthma drugs. The contribution of altered airway smooth muscle function to this untoward effect has been investigated.

Guinea-pigs (450-600 gm) were sensitised by intraperitoneal injection (1 ml) of a suspension containing ovalbumin (OA, 10 ug/ml) and aluminium hydroxide (10 mg/ml) and separately with pertussis toxin (0.25 ml) on day 0, boosted on day 14 and implanted with either saline (C) or salbutamol (S) (1 mg/kg/day, Alzet minipump, s.c.) between day 21 and day 30. Six days later animals were anaesthetised with phenobarbitone (100 mg/kg i.p.) and pentobarbitone (30 mg/kg i.p.) paralysed with gallamine (10 mg/kg i.m.) and ventilated (1 hz, 5 ml/kg) via a tracheal cannula. Airway resistance (R_L cm H_2O/l/sec) and compliance (C_{dyn} ml H_2O/l/sec) were calculated from measurement of tracheal airflow and transpulmonary pressure (Digital electronic pulmonary monitoring system, Mumed Ltd., U.K.). Animals were challenged with aerosolised OA (10-1000 ug/ml for 10 min) and changes in R_L and C_{dyn} were monitored at each breath. Airway responses to inhaled OA or intravenous histamine (1.0 & 1.8 ug/kg) were expressed as the maximal increase in R_L (mean±sem).

Responses to histamine in naive animals (107±67, 198±77, n=4) were not dissimilar from C animals (109±48, 262±91, n=10). Prior treatment with S (1 mg/kg/day s.c.) resulted in a slight reduction of these responses (46±12, 139±42, n=10, NS). No response to inhaled OA (100 ug) was observed in naive animals, in contrast to C animals (132±38, n=10) which developed increased reactivity to histamine following antigen challenge (418±64, 799±76, n=10). In animals pretreated with S, the reaction to antigen (334±58, n=10) was significantly (P<0.001) increased, even though airway responses to histamine were slightly reduced (225±66, 613±106, n=10).

The present results demonstrate that pretreatment of sensitised guinea-pigs with S augments the response to antigen. Altered distribution or increased dosage of inhaled allergen, altered airway reactivity or hypoxic vasoconstriction are mechanisms that might contribute to this phenomenon.

Brit. J. Pharmacol. 99, 66P (1990)

ATTACHMENT C

United States Patent [19]
Sunshine et al.

[11] Patent Number: 4,975,426
[45] Date of Patent: Dec. 4, 1990

[54] COUGH/COLD MIXTURES COMPRISING NON-SEDATING ANTIHISTAMINE DRUGS

[75] Inventors: Abraham Sunshine, New York; Eugene M. Laska, Larchmont; Carole E. Siegel, Mamaroneck, all of N.Y.

[73] Assignee: Analgesic Associates, Larchmont, N.Y.

[21] Appl. No.: 315,161

[22] Filed: Feb. 24, 1989

Related U.S. Application Data

[62] Division of Ser. No. 59,635, Jun. 8, 1987, Pat. No. 4,829,064.

[51] Int. Cl.[5] A61K 31/60; A61K 31/62; A61K 31/615; A61K 31/505; A61K 31/44; A61K 31/445; A61K 31/19

[52] U.S. Cl. 514/159; 514/161; 514/165; 514/166; 514/256; 514/290; 514/315; 514/336; 514/570

[58] Field of Search 514/159, 165, 256, 290, 514/315, 336, 570, 629, 630

[56] References Cited

PUBLICATIONS

Handbook of Nonprescription Drugs 8th ed. (1986) pp. 137–139 and 166.
The Merck Index 10th ed (1983), pp. 1310–1311 and APP-1.
Chemical Abstracts 104(1):517, Cohen et al. (1986).
Chemical Abstracts 106(5):27517t, Roman et al. (1986).
Chemical Abstracts, 106(15):113384d, Calcutt et al. (4/13/87).

Primary Examiner—Douglas W. Robinson
Assistant Examiner—Raymond J. Henley, III
Attorney, Agent, or Firm—Burns, Doane, Swecker & Mathis

[57] ABSTRACT

Pharmaceutical compositions and methods of using same comprising aspirin, sodium salicylate, salicylamide or acetaminophen, in combination with a non-sedating antihistamine and optionally one or more other active components selected from a decongestant, cough suppressant (antitussive) or expectorant are provided for the relief of cough, cold, cold-like and/or flu symptoms and the discomfort, pain, headache, fever and general malaise associated therewith.

33 Claims, No Drawings

ATTACHMENT D

United States Patent [19]
Sunshine et al.

[11] Patent Number: 4,923,898
[45] Date of Patent: May 8, 1990

[54] ANALGESIC, ANTI-INFLAMMATORY AND SKELETAL MUSCLE RELAXANT COMPOSITIONS COMPRISING NON-STEROIDAL ANTI-INFLAMMATORY DRUGS AND MUSCULOSKELETAL RELAXANTS AND METHODS OF USING SAME

[75] Inventors: Abraham Sunshine, New York; Eugene M. Laska, Larchmont; Carole E. Siegel, Mamaroneck, all of N.Y.

[73] Assignee: Analgesic Associates, Larchmont, N.Y.

[21] Appl. No.: 227,989

[22] Filed: Aug. 3, 1988

Related U.S. Application Data

[60] Division of Ser. No. 114,751, Oct. 30, 1987, Pat. No. 4,780,463, which is a division of Ser. No. 815,502, Jan. 2, 1986, Pat. No. 4,722,938, which is a continuation of Ser. No. 686,380, Dec. 26, 1984, abandoned.

[51] Int. Cl.[5] A61K 31/19
[52] U.S. Cl. 514/557
[58] Field of Search 514/557

[56] References Cited

U.S. PATENT DOCUMENTS

4,683,243 7/1987 Sunshine et al. 514/557

FOREIGN PATENT DOCUMENTS

2121529 8/1972 France .

OTHER PUBLICATIONS

Physicians' Desk Reference, 28th Ed., (1974), p. 972.
Armas & Valencia, "Eficacia terapeutica de la asociacion naproxen-carisoprodol en ciertas enfermedader musculoesqueleticas", [Therapeutic Effectiveness of Naproxen-Carisoprodol Association in Certain Musculoskeletal Disorders], in Investigation Medica Interacional, pp. 350–356, (1983), and English translation thereof.
Goti & Valenica, "Caracterizacion clinica de una nueva asociacion (naproxen+carisprodol) en padecimientos del aparato musculoesqueletico", [Clinical Description of a New Association (Naproxen+Carisoprodol) in Ailments of the Musculoskeletal Apparatus], *Investigation Medica Internacional*, pp. 475–478, (1983), and English translation thereof.
Socialist Republic of Romania Description of Invention 82,717, copy of patent and English translation thereof.
Rego, "Mio-Relaxantes No Tratamento Das Lombalgias Aguda E Da Lombo-Ciaticas Recentes", [Muscle Relaxants in the Treatment of Acute Lumbalgias and Recent Lumbo-Sciatica Cases], *Acta Reumatolgica Portuguesa*, II, 2:363–364, (1974), copy of the original and English translation thereof.
Schror, "Analgetisch-antiphlogistische Therapie Von Schmerzzustanden des Bewegungsapparates", [Analgesic-Antiphlogistic Therapy of Locomotor System Pain], *Therapiewoche*, 28, 5657–5663, (1978), copy of the original and English translation thereof.
Schar, "Medikamentose Behandlung von Lumboischialgien", [Drug Treatment of the Lumbago-Sciatic Syndrome], Schweiz. Rundschau Med., (Praxis), vol. 68, No. 5, pp. 141–142, (Jan. 30, 1979), copy of original article and English translation thereof.
Kolodny and Klipper, "Bone and Joint Diseases in the Elderly", *Hospital Practice*, pp. 91–101, (Nov., 1976).
Nascimento, "Use of an Association Containing an Analgesic, a Muscle Relaxant and Vitamin B Complex in Degenerative Joint Diseases, Extra-Articular Rheumatic Ailments and Traumatic Afflictions", *F med* (BR), 83(3):361–363, (1981), original article and English translation thereof
Repschlaeger and McPherson, "Classification, Mechanism and Management of Headache", *Clinical Pharmacy*, vol. 3, pp. 139–150, (Mar.–Apr. 1984).

Primary Examiner—Stanley J. Friedman
Attorney, Agent, or Firm—Burns, Doane, Swecker & Mathis

[57] ABSTRACT

Novel pharmaceutical analgesic, anti-inflammatory and skeletal muscle relaxant compositions and methods of using same comprising an analgesically and anti-inflammatory effective amount of at least one non-steroidal anti-inflammatory drug other than aspirin, acetaminophen and phenacetin, in combination with an effective amount of a skeletal muscle relaxant.

20 Claims, No Drawings

ATTACHMENT E

United States Patent [19]
Sunshine et al.

[11] Patent Number: 5,025,019
[45] Date of Patent: Jun. 18, 1991

[54] COUGH/COLD MIXTURES COMPRISING NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

[75] Inventors: Abraham Sunshine, New York; Eugene M. Laska, Larchmont; Carole E. Siegal, Mamaroneck, all of N.Y.

[73] Assignee: Analgesic Associates, Larchmont, N.Y.

[21] Appl. No.: 438,074

[22] Filed: Nov. 20, 1989

Related U.S. Application Data

[62] Division of Ser. No. 144,099, Jan. 15, 1988, Pat. No. 4,920,149, which is a division of Ser. No. 887,203, Jul. 21, 1986, Pat. No. 4,738,966, which is a division of Ser. No. 752,546, Jul. 8, 1985, Pat. No. 4,619,934, which is a division of Ser. No. 598,502, Apr. 9, 1984, Pat. No. 4,552,899.

[51] Int. Cl.[5] A61K 31/19; A61K 31/44; A61K 31/435; A61K 31/445

[52] U.S. Cl. 514/277; 514/290; 514/325; 514/568; 514/653

[58] Field of Search 514/568, 653, 277, 290, 514/325

Primary Examiner—Stanley J. Friedman
Attorney, Agent, or Firm—Burns, Doane, Swecker & Mathis

[57] ABSTRACT

Pharmaceutical compositions and methods of using same comprising a non-steroidal anti-inflammatory drug in combination with at least one other active component selected from an antihistamine, decongestant, cough suppressant (antitussive) or expectorant are provided for the relief of cough, cold and cold-like symptoms.

23 Claims, No Drawings

ATTACHMENT F

0701.027

N

s substrate for
n: Biological

I. (1986).Tissue
ogenous substrate
inase. Endocrino-

ma virus transfor-
known polypeptide

tyl and palmityl
cal Chemistry.262-

t liver membranes
substrate for the
epidermal growth

apidly stimulates
ntact cells.Nature

icher E.,Obermaier
a 46-KDa membrane
lation of several
e National Academy

.C.,Wang P.H.,Kahn
type I insulin
C/ III Interna-
.k ad.
R.W.,Taylor S.I.
oprotein substrate
e in inatact H-35
of Science .U.S.A.

THE β-ADRENERGIC RECEPTOR KINASE: ROLE IN HOMOLOGOUS DESENSITIZATION IN S49 LYMPHOMA CELLS

Ruth H. Strasser, Jeffrey L. Benovic,
Robert J. Lefkowitz and Marc G. Caron

Howard Hughes Medical Institute
Departments of Medicine, Biochemistry and Physiology
Duke University Medical Center
Durham, North Carolina 27710 USA

Summary

Phosphorylation of the β-adrenergic receptor (βAR) is closely associated with homologous desensitization of the β-adrenergic receptor-coupled adenylate cyclase system. Homologous desensitization and receptor phosphorylation also occur in cell mutants which are deficient in their cAMP-dependent protein kinase (kin^- mutant of S49 lymphoma cells). βAR phosphorylation is mediated by a cAMP-independent protein kinase which phosphorylates the receptor only when it is occupied by a β-agonist. During the time course of desensitization the βAR kinase (βARK) activity is translocated from a cytoplasmic to a plasma membrane location. βARK translocation can also be effected by prostaglandin E_1 (PGE_1) suggesting that this βARK may represent a more general enzyme capable of phosphorylating other adenylate cyclase-coupled receptors. Thus, βARK may play a key role in the process of homologous desensitization of adenylate cyclase coupled receptors.

Extracellular hormones interact with specific receptors at the outer surface of the plasma membrane and thus initiate a cellular response. One of the best studied transmembrane signalling systems known to be coupled to the occupancy of cell surface receptors is adenylate cyclase. The adenylate cyclase system is composed of various components all of which have been purified to homogeneity (Shorr et al., 1982; Homcy et al., 1983; Benovic et al., 1984; Codina et al., 1984; Northup et al., 1980; Sternweis et al., 1981; Bokoch et al., 1984; Pfeuffer et al., 1985). Initially, agonist binding to the receptor promotes coupling of the occupied receptor to one of the guanine nucleotide binding regulatory proteins. These proteins are members of a

Adv. Exp. Med. Biol. 231, 503-517 (1988)

EXHIBIT 1

family of heterotrimeric proteins consisting of α, β and γ subunits. Stimulatory receptors like the β-adrenergic (Cerione et al., 1984) or glucagon (Iyengar et al., 1979) receptors couple to the stimulatory regulatory protein N_s (or G_s) whereas inhibitory receptors like the α_2-adrenergic (Jacobs et al., 1976) or M_2-muscarinic (Harden et al., 1982) receptors couple to the inhibitory regulatory protein N_i (or G_i).

Prolonged exposure to agonist hormones, either stimulatory or inhibitory, results in an attenuation of the response to the hormonal activation, a phenomenon called tachyphylaxis or desensitization (Harden, 1983; Sibley and Lefkowitz, 1985; Sharma et al., 1975). One of the best studied models for desensitization is the β-adrenergic receptor-coupled adenylate cyclase system. In this system two different forms of desensitization have been characterized. Homologous or hormone-specific desensitization results in an attenuated response only to the desensitizing hormone. In contrast, the heterologous form of desensitization leads to a general decrease of adenylate cyclase activity promoted not only by the desensitizing hormone but by other hormones and non-hormonal stimulators as well.

Previous studies have demonstrated that phosphorylation of the β-adrenergic receptor is involved in the mechanism of heterologous desensitization (Stadel et al., 1983; Sibley et al., 1984). In this form of desensitization phosphorylation of the β-adrenergic receptor is at least in part cAMP-dependent and mediated by the cAMP-dependent protein kinase (protein kinase A) (Strulovici et al., 1984; Sibley et al., 1984; Benovic et al., 1985).

Homologous desensitization, however, appears to be independent of cAMP since it has been observed in systems which are defective in their cAMP-dependent pathway (Green and Clark, 1981; Green et al., 1981; Perkins, 1983; Clark et al., 1985). These systems either lack the N_s protein or a functional cAMP-dependent protein kinase. Consequently β-adrenergic receptor occupancy does not result in an increase in intracellular cAMP levels (cyc^- mutant of S49 lymphoma cells) (Bourne et al., 1975; Bourne et al., 1981; Ross and Gilman, 1977) or cAMP-dependent protein phosphorylation (kin^- mutant of S49 lymphoma cells) (Steer et al., 1976; Steinberg et al., 1978; Mahan et al., 1985). Therefore, if phosphorylation of the β-adrenergic receptor is involved in the process of homologous desensitization it must be catalyzed by a non cAMP-dependent protein kinase. To address these questions we utilized the kin^- mutant of the S49 lymphoma cells (Steer et al., 1976; Steinberg et al., 1978; Mahan et al., 1985). We document here a cAMP independent pathway of β-adrenergic receptor active phosphorylation during homologous desensitization. The kinase involved in this phosphorylation

process is distinct from other known kinases and phosphorylates only the agonist occupied form of the β-adrenergic receptor. Moreover, during desensitization the cytosolic kinase activity becomes transiently translocated to the plasma membranes in a cAMP-independent manner.

MATERIALS AND METHODS

Cells and incubations – S49 lymphoma cells, wild type (clone 24.3.2) and kin$^-$ mutants (clone 25.6.1), were grown in Dulbecco's modified Eagle's medium with 10% horse serum. Cells were harvested by centrifugation (800 x g, 3 min), washed three times with phosphate-free Dulbecco's modified Eagle's medium and incubated at 37°C for various periods of time (as indicated) in the presence of a β-adrenergic agonist for desensitization. To study the in situ phosphorylation of the β-adrenergic receptor the intracellular pool of ATP was labeled by incubating the cell with carrier-free ^{32}P (0.3 mCi/ml) prior to desensitization. The desensitization incubation was stopped by adding ice-cold phosphate-buffered saline with propranolol (10^{-6} M) followed by immediate sedimentation of the cells (800 x g, 5 min).

Purification of the β-adrenergic receptor – The purification of the in situ phosphorylated β-adrenergic receptor was performed by affinity chromatography as previously described (Strasser et al., 1986a). Purified β-adrenergic receptor from hamster lung (Benovic et al., 1984) was used as a substrate for the receptor kinase assays.

Preparation of cell fractions for assay of β-adrenergic receptor kinase – After incubation (as described above) the sedimented cells were lysed in 2 volumes of 10 mM Tris, 15 mM $MgCl_2$, 5 mM EDTA, 10^{-4} M PMSF, 5 µg/ml leupeptin, 5 µg/ml pepstatin, pH 7.4 using a glass homogenizer (20 strokes). Unbroken cells and cell nuclei were sedimented at 800 x g for 10 min and discarded. The plasma membranes were then sedimented at 48,000 x g for 20 min. To obtain a cytosolic fraction the 48,000 x g supernatant was centrifuged at 150,000 x g for 60 min. To test for the receptor kinase activity the cytosolic and plasma membrane fractions were used directly.

Kinase assay – Pure β-adrenergic receptor was reconstituted into phospholipid vesicles as previously described (Benovic et al., 1986). The reconstituted β-adrenergic receptor (≈ 5 pmol) was incubated in 25 mM Tris, 10 mM NaCl, 1.5 mM EDTA, 1 mM EGTA, 5 mM $MgCl_2$, 5 mM NaF, 50 µM Na_3VO_4, 10^{-4} M PMSF, 5 µg/ml leupeptin, 5 µg/ml pepstatin, pH 7.4 in the presence of 50 µM [γ-^{32}P]ATP (25 cpm/fmol), with or without 10^{-4} M isoproterenol or the β antagonist alprenolol (10^{-5} M) and in the presence of the appropriate kinase preparation for 20 min at 30°C in a

DLEV012136

total volume of 100 µl. The reaction was stopped by adding 1 ml of ice-cold 100 mM NaCl, 10 mM Tris, 2% digitonin, pH 7.2. The β-adrenergic receptor was then repurified by affinity chromatography (Benovic et al. 1986).

Other assays - β-Adrenergic receptor assays, adenylate cyclase assays and $NaDodSO_4$/polyacrylamide gel electrophoresis were performed essentially as described in Strasser et al. (1986a).

RESULTS

Wild type (WT) and kin^- mutants of the S49 lymphoma cells preincubated with carrier-free [^{32}P]Pi to label the intracellular ATP pool (Strasser et al., 1986a), were incubated in the presence of 10^{-4} M isoproterenol to induce desensitization. Homologous desensitization (agonist specific) was documented by measuring the adenylate cyclase activity in the plasma membranes (data not shown). As shown in Fig. 1 homologous desensitization induces a dramatic increase in the phosphorylation of the β-adrenergic receptor of both the wild type and the kin^- mutant of the S49 lymphoma cells (0.2 mol P/mol β-adrenergic receptor for control and 0.8 mol P/mol for desensitized cells). These results indicate that a non cAMP-dependent pathway is involved in the phosphorylation process of the β-adrenergic receptor during homologous desensitization.

To identify the kinase activity which is involved in this phosphorylation process, the cytoplasmic and plasma membrane fractions from untreated kin^- mutants of the S49 lymphoma cells were tested for their ability to phosphorylate pure βAR reconstituted into phospholipid vesicles. As shown in Fig. 2 cytoplasmic fractions of these cells phosphorylate the βAR but only in the presence of the β-agonist isoproterenol. The presence of the β-agonist induces about a 5- to 10-fold increase in the phosphorylation of the βAR. The effect of the agonist can be completely blocked by the β antagonist alprenolol. These data indicate that in the reconstituted system agonist occupancy of the βAR induces a state of the receptor which makes it a much better substrate for βARK activity present in the cytosol of these cells. This effect of agonist is independent of the generation of cAMP or presumably any other unknown second messenger since the effect is observed in an in vitro system utilizing purified components.

As mentioned above the β-adrenergic receptor kinase is a predominantly cytosolic enzyme. Yet the β-adrenergic receptor is an integral membrane glycoprotein (Stiles et al., 1984). Thus, the question arises as to how does a cytosolic enzyme function to

ding 1 ml of
The
hromatography

late cyclase
were performed

a cells
racellular ATP
esence of 10^{-4} M
sensitization
ylate cyclase
shown in Fig. 1
in the
e wild type and
1 β-adrenergic
cells). These
nv d in the
ri. omologous

in this
brane fractions
ere tested for
nto phospholipid
these cells
-agonist
bout a 5-to 10-
ffect of the
lprenolol. These
occupancy of the
ch better
hese cells. This
AMP or presumably
observed in an in

e is a
eceptor is an
Thus, the
io

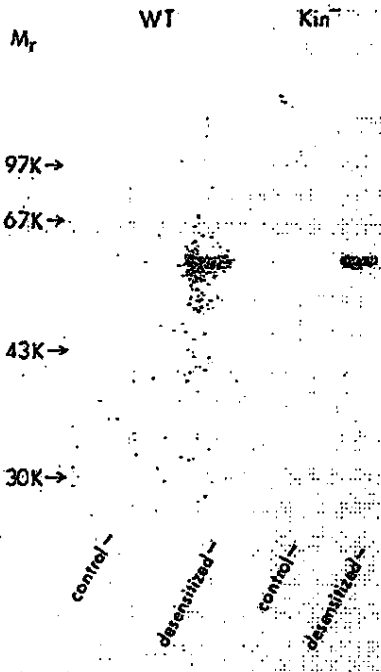


Fig. 1. Phosphorylation of the β-adrenergic receptor during desensitization in WT and kin⁻ S49 lymphoma cells. Wild type and kin⁻ mutants of the S49 lymphoma cells were incubated (37°C) with 0.3 mCi $^{32}P_i$/ml Pi as described in Methods. Desensitization was induced by incubating the cells with isoproterenol (10^{-5} M) for 20 min. The β-adrenergic receptors were purified and visualized by autoradiography after gel electrophoresis (see Methods). Indicated on the left is the relative mobility of the molecular weight standards. Indicated on the right (arrow) is the relative mobility of the β-adrenergic receptors derived either from control (lane 1) or desensitized (lane 2) wild type cells or control (lane 3) or desensitized (lane 4) kin⁻ mutant cells.

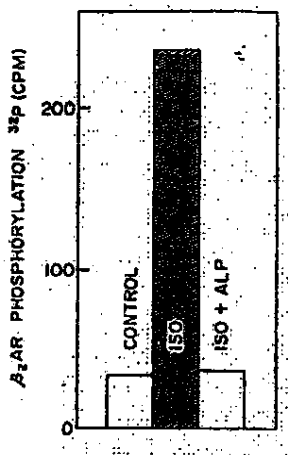


Fig. 2. Influence of agonist occupancy on phosphorylation of the β-adrenergic receptor by the β-adrenergic receptor kinase. Pure hamster lung β-adrenergic receptor was reconstituted into lipid vesicles and incubated for 30 min at 30°C with crude β-adrenergic receptor kinase prepared from a kin⁻ cell cytosol fraction. The incubations also contained either no ligand (control), 100 μM (-)isoproterenol (Iso) or 100 μM (-)isoproterenol + 10 μM (±)alprenolol (Iso + Alp). Phosphorylated β-adrenergic receptor was then repurified, electrophoresed on a 10% polyacrylamide gel and visualized by autoradiography (see Methods).

DLEV012139

phosphorylate a plasma membrane protein? In an attempt to answer this question we followed cytoplasmic enzyme activity and in situ phosphorylation of the β-adrenergic receptor as a function of time of exposure to isoproterenol. As the β-adrenergic receptors become rapidly phosphorylated, the β-adrenergic receptor kinase activity rapidly disappears from the cytosolic fraction (Fig. 3). After 15 min of

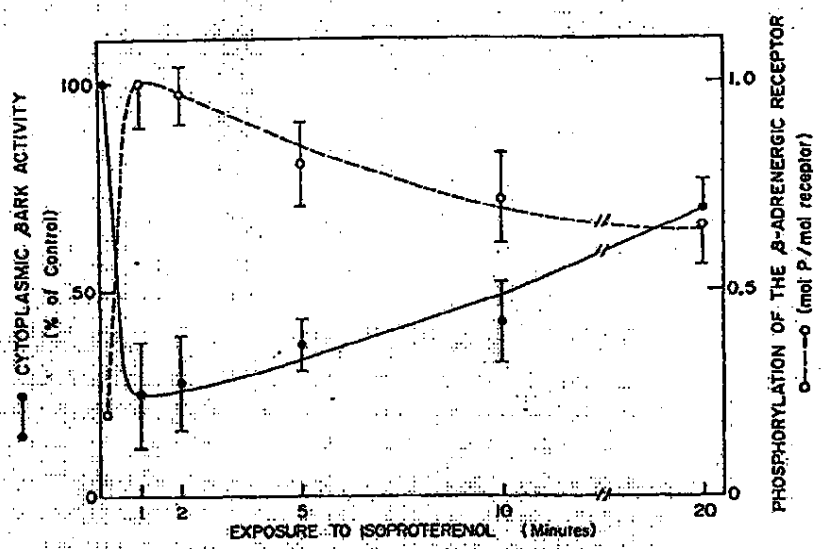


Fig. 3. Time course of cytoplasmic βARK activity and in situ β-adrenergic receptor phosphorylation during desensitization. Kin⁻ mutants of the S49 lymphoma cells were incubated 0-30 min in the presence of 10^{-5} M isoproterenol to induce homologous desensitization. The β-adrenergic receptor kinase activity relative to control (●) was measured using the reconstituted, agonist occupied hamster lung receptor as substrate (see Methods). The phosphorylation of the β-adrenergic receptor (○) within the plasma membrane of the intact cells (in situ) was quantitated after autoradiography of the purified receptor (see Methods).

ho tion of the
na Pure hamster
id vesicles and
receptor kinase
bations also
otarenol (Iso) or
Alp).
ied,
lized by

isoproterenol induced desensitization about 75% of the kinase activity has vanished from the cytosol (Fig. 3). This decrease in cytosolic kinase activity is accompanied by a simultaneous increase in the kinase activity associated with the plasma membrane. As shown in Fig. 4, an increase in membrane activity of about 6.5 fold can be observed indicating that the β-adrenergic receptor kinase is translocated from the cytosol to the plasma membrane upon β agonist promoted desensitization. At longer times (Fig. 3) (20-60 min) when the extent of phosphorylation of the total pool of receptor decreases the cytosolic kinase activity returns to control levels (data not shown).

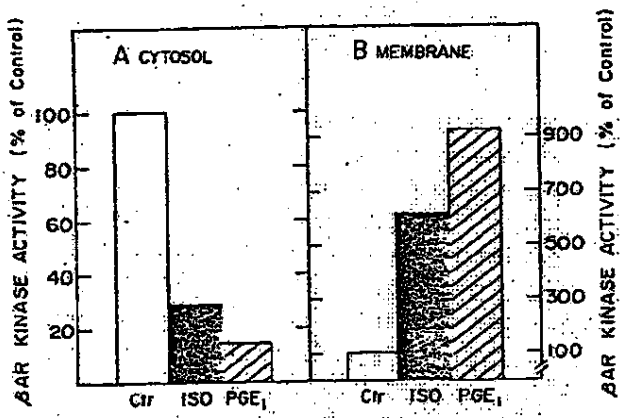


Fig. 4. Translocation of the β-adrenergic receptor kinase from the cytosol to the plasma membrane. Kin^- mutants of the S49 lymphoma cells were desensitized for 15 min with 10^{-5} M isoproterenol (ISO) or 10^{-6} M prostaglandin E_1. The β-adrenergic receptor kinase activity was measured in the cytoplasmic (cytosol) and in the plasma membrane (membrane) fractions using the reconstituted, agonist occupied β-adrenergic receptor as substrate (see Methods). Indicated are the relative kinase activities compared to controls.

These data suggests that specific agonist occupancy of the β-adrenergic receptor triggers the translocation of the receptor kinase. We next wished to determine whether this kinase is a specific β-receptor kinase or whether it is an enzyme with more general substrate specificity. Since the β-adrenergic receptor is the only adenylate

DLEV012141